Regional Oral History Office
The Bancroft Library

University of California
Berkeley, California

Program in the History of the Biosciences and Biotechnology

Horace A. Barker, Ph.D.

SCIENTIST AND PROFESSOR OF MICROBIAL BIOCHEMISTRY AT BERKELEY

With an Introduction by
Clinton E. Ballou, Ph.D.

Interviews Conducted by
Sally Smith Hughes, Ph.D.
in 1998 and 1999

Since 1954 the Regional Oral History Office has been interviewing leading participants in or well-placed witnesses to major events in the development of northern California, the West, and the nation. Oral history is a method of collecting historical information through tape-recorded interviews between a narrator with firsthand knowledge of historically significant events and a well-informed interviewer, with the goal of preserving substantive additions to the historical record. The tape recording is transcribed, lightly edited for continuity and clarity, and reviewed by the interviewee. The corrected manuscript is indexed, bound with photographs and illustrative materials, and placed in The Bancroft Library at the University of California, Berkeley, and in other research collections for scholarly use. Because it is primary material, oral history is not intended to present the final, verified, or complete narrative of events. It is a spoken account, offered by the interviewee in response to questioning, and as such it is reflective, partisan, deeply involved, and irreplaceable.

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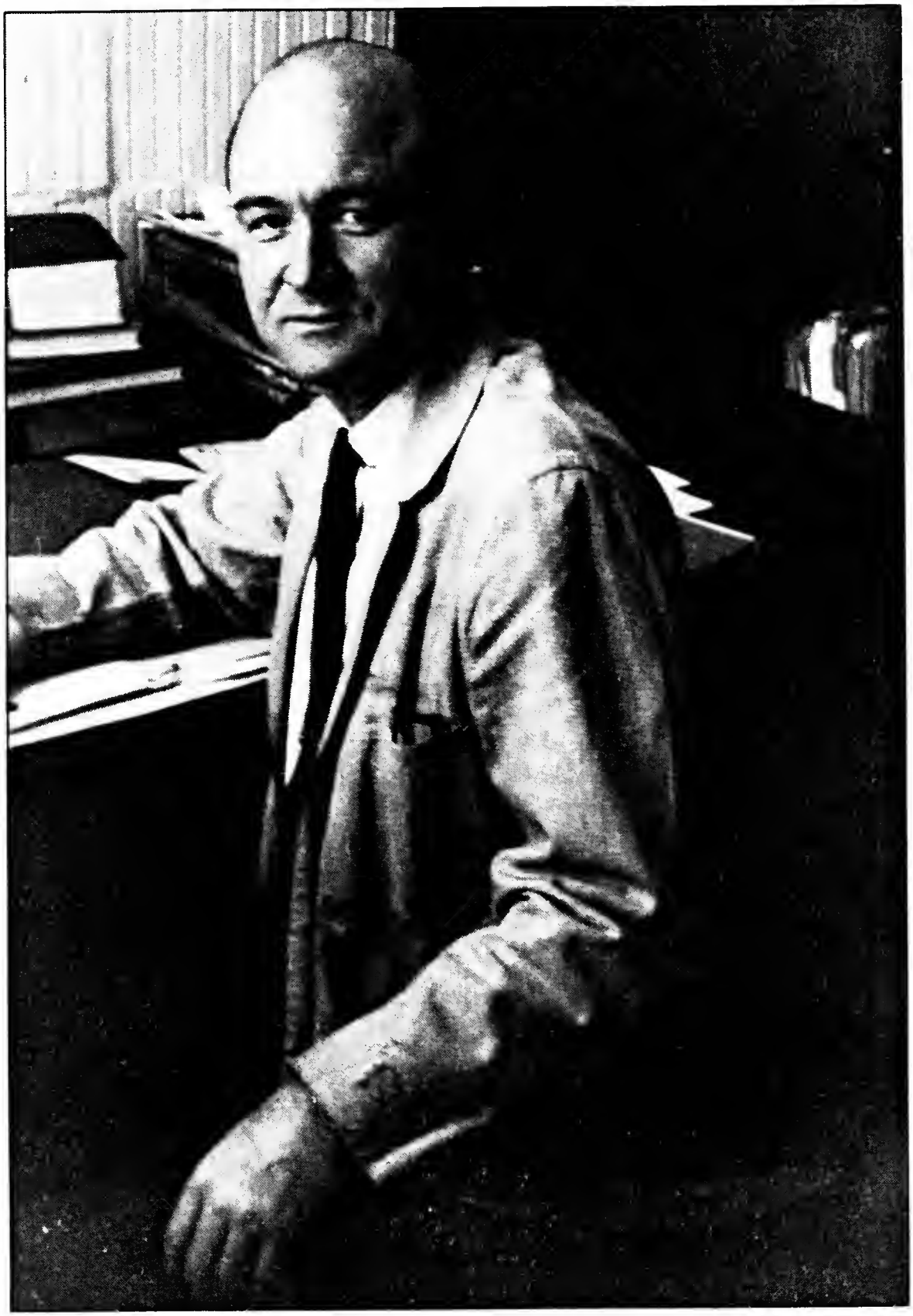

Horace Albert Barker

New York Times, January 10, 2001.

Horace Barker, 93, Scientist Who Studied Body Chemistry

By WOLFGANG SAXON

Dr. Horace Albert Barker, a microbiologist and biochemist who helped to unravel the complex processes of chemical conversion inside living organisms, died on Dec. 24 at his home in Berkeley, Calif. He was 93.

Dr. Barker made his mark in the 1950's by tracing the biochemical function of vitamin B12. His investigation helped explain complex ways chemistry works in the body.

Earlier, in 1944, he was on a team of researchers that detected the role of enzymes when living cells synthesize sucrose. The researchers gained that insight with one of the earliest laboratory uses of radioactive carbon-14 tracers; a technique Dr. Barker helped pioneer.

For his work, specifically that involving vitamin B12, Dr. Barker received one of the 12 National Medals of Science for 1968 from President Lyndon B. Johnson.

Born in Oakland, Calif., Horace Barker graduated in 1929 from Stanford University, where he also received a Ph.D. in chemistry four years later.

Dr. Barker became an instructor in soil microbiology at the University of California in 1936. He was named a professor in the department of biochemistry when it was set up in 1959 and served as chairman in the 1960's.

Jane Scherr, 1992

Dr. Horace A. Barker

Dr. Barker's studies dealt with vitamin B12 coenzymes, vitamin chemistry, bacterial metabolism, fatty acid oxidation and synthesis, carbohydrate transformations, and amino acid and purine metabolism. Together, the studies helped build a foundation for much of what is known today of metabolism and its role in sickness and health.

Working with a common soil bacterium dredged from the mud of San Francisco Bay, Dr. Barker led a research team that in 1959 discovered vitamin B12 coenzyme, an active form of vitamin B12, deployed in vital chemical conversions in the body. He then mapped out many of the metabolic reactions this entails.

In doing so, Dr. Barker clarified the vitamin B12 coenzyme's role in building body tissue. This in turn contributed to the understanding of several diseases, including pernicious anemia caused by a deficiency of vitamin B12.

Dr. Barker wrote or helped write some 230 scientific publications. Among his honors was his election to the National Academy of Sciences and to the American Academy of Arts and Sciences.

Dr. Barker is survived by two daughters, Barbara B. Friede, of Piedmont, Calif., and Elizabeth F. Mark, of Lexington, Mass.; a son, Robert H., of Camino, Calif.; and four grandchildren. His wife, Margaret McDowell Barker, died in 1995 after 62 years of marriage.

Dr. Barker retired with emeritus status in 1975 but remained active in the biochemistry department at Berkeley well after turning 80.

Cataloguing information

Horace A. Barker, Ph.D. (1907-2000) Professor of Biochemistry

Scientist and Professor of Microbial Biochemistry at Berkeley, 2001, xix, 118 pp.

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Introduction by Clinton E. Ballou, Ph.D., Professor Emeritus, Biochemistry, UC Berkeley.

Interviewed 1998-1999 by Sally Smith Hughes for the Program in the History of Biosciences and Biotechnology, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 2001.

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BIOTECHNOLOGY SERIES HISTORY--Sally Smith Hughes, Ph.D.

Genesis of the Program in the History of the Biological Sciences and Biotechnology

In 1996, a long-held dream of The Bancroft Library came true with the launching of its Program in the History of the Biological Sciences and Biotechnology. For years, Bancroft had wished to document the history of the biological sciences on the Berkeley campus, particularly its contributions to the development of molecular biology. Bancroft has strong holdings in the history of the physical sciences--the papers of E.O. Lawrence, Luis Alvarez, Edwin McMillan, and other campus figures in physics and chemistry, as well as a number of related oral histories. These materials support Berkeley's History of Science faculty, as well as scholars from across the country and around the world.

Although the university is located next to the greatest concentration of biotechnology companies in the world, Bancroft had no coordinated program to document the industry nor its origins in academic biology. For a decade, the staff of the Regional Oral History Office had sought without success to raise funds for an oral history program to record the development of the industry in the San Francisco Bay Area. When Charles Faulhaber arrived in 1995 as Bancroft's new director, he agreed to the need to establish a Bancroft program to capture and preserve the collective memory and papers of university and corporate scientists and the pioneers who created the biotechnology industry. He too saw the importance of documenting the history of a science and industry which influences virtually every field of the life sciences, generates constant public interest and controversy, and raises serious questions of public policy. Preservation of this history was obviously vital for a proper understanding of science and business in the late twentieth century.

Bancroft was the ideal location to launch such an historical endeavor. It offered the combination of experienced oral history and archival personnel, and technical resources to execute a coordinated oral history and archival program. It had an established oral history series in the biological sciences, an archival division called the History of Science and Technology Program, and the expertise to develop comprehensive records management plans to safeguard the archives of individuals and businesses making significant contributions to molecular biology and biotechnology. It also had longstanding cooperative arrangements with UC San Francisco and Stanford University, the other research universities in the San Francisco Bay Area. The history of biotech project was to provide a basis for continuing collaboration among the three institutions in the documentation of recent science and technology through oral history and archival collection. The only ingredient missing was funding.

In April 1996, the dream became reality. Daniel E. Koshland, Jr. provided seed money for a center at The Bancroft Library for historical research on the biological sciences and biotechnology. Thanks to this generous gift, Bancroft has begun to build an integrated collection of research materials--primarily oral history transcripts, personal papers, and archival collections--related to the history of the biological sciences and biotechnology in university and industry settings. One of the first steps was to create a board composed of distinguished figures in academia and industry who advise on the direction of the oral history and archival components. The Program's initial concentration is on the San Francisco Bay Area and northern California. But its ultimate aim is to document the growth of molecular biology as an independent field of the life sciences, and the subsequent revolution which established biotechnology as a key contribution of American science and industry.

UCSF Library, with its strong holdings in the biomedical sciences, is a collaborator on the archival portion of the Program. David Farrell, Bancroft's curator of the History of Science and Technology, serves as liaison. In February 1998, Robin Chandler, head of UCSF Archives and Special Collections, completed a survey of corporate archives at local biotechnology companies and document collections of Berkeley and UCSF faculty in the biomolecular sciences. The ultimate aim is to ensure that personal papers and business archives are collected, cataloged, and made available for scholarly research.

Project Structure

With the board's advice, Sally Hughes, a science historian at the Regional Oral History Office, began lengthy interviews with Robert Swanson, a co-founder and former CEO of Genentech in South San Francisco; Arthur Kornberg, a Nobel laureate at Stanford; and Paul Berg, also a Stanford Nobel laureate. A short interview was conducted with Niels Reimers of the Stanford and UCSF technology licensing offices. These oral histories build upon ones conducted in the early 1990s, under UCSF or Stanford auspices, with scientists at these two universities.[1] The oral histories offer a factual, contextual, and vivid personal history that enriches the archival collection, adding information that is not usually present in written documents. In turn, the archival collections support and provide depth to the oral history narrations.

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[1]Hughes conducted oral histories with Herbert Boyer, William Rutter, and Keith Yamamoto of UCSF, and with Stanley Cohen of Stanford. To date, the first volume of the oral history with Dr. Rutter is available at the Bancroft and UCSF libraries; transcripts of the other interviews are currently under review by the interviewees.

Primary and Secondary Sources

This oral history program both supports and is supported by the written documentary record. Primary and secondary source materials provide necessary information for conducting the interviews and also serve as essential resources for researchers using the oral histories. The oral histories also orient scholars unfamiliar with the field or the scientist to key issues and participants. Such orientation is particularly useful to a researcher faced with voluminous, scattered, and unorganized primary sources. This two-way "dialogue" between the documents and the oral histories is essential for valid historical interpretation.

Beginning with the first interviews in 1992, the interviewer has conducted extensive documentary research in both primary and secondary materials. She gratefully acknowledges the generosity of the scientists who have made their personal records available to her: Paul Berg, Stanley Cohen, Arthur Kornberg, William Rutter, and Keith Yamamoto. She also thanks the archivists at Bancroft, UCSF, and Stanford libraries, and personnel at Chiron, Genentech, and Stanford's Office of Technology Licensing, for assistance in using archival collections.

Oral History Process

The oral history methodology used in this program is that of the Regional Oral History office, founded in 1954 and producer of over 1,600 oral histories. The method consists of research in primary and secondary sources; systematic recorded interviews; transcription, light editing by the interviewer, and review and approval by the interviewee; library deposition of bound volumes of transcripts with table of contents, introduction, interview history, and index; cataloging in UC Berkeley and national online library networks (MELVYL, RLIN, and OCLC); and publicity through ROHO news releases and announcements in scientific, medical, and historical journals and newsletters and via the ROHO and UCSF Library Web pages.

Oral history as a historical technique has been faulted for its reliance on the vagaries of memory, its distance from the events discussed, and its subjectivity. All three criticisms are valid; hence the necessity for using oral history documents in conjunction with other sources in order to reach a reasonable historical interpretation.[1] Yet these acknowledged weaknesses of oral history, particularly its subjectivity, are also its strength. Often individual perspectives provide information unobtainable through more traditional sources. Oral history in skillful hands provides the context in which events occur--the social, political, economic, and

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[1]The three criticisms leveled at oral history also apply in many cases to other types of documentary sources.

institutional forces which shape the course of events. It also places a personal face on history which not only enlivens past events but also helps to explain how individuals affect historical developments.

An advantage of a series of oral histories on a given topic, in this case molecular biology and biotechnology, is that the information each contains is cumulative and interactive. Through individual accounts, a series can present the complexities and interconnections of the larger picture. Thus the whole (the series) is greater than the sum of its parts (the individual oral histories), and should be considered as a totality.

Emerging Themes

Although the oral history program is still in its infancy, several themes are emerging. One is "technology transfer," the complicated process by which scientific discovery moves from the university laboratory to industry where it contributes to the manufacture of commercial products. The oral histories show that this trajectory is seldom a linear process, but rather is influenced by institutional and personal relationships, financial and political climate, and so on.

Another theme is the importance of personality in the conduct of science and industry. These oral histories testify to the fact that who you are, what you have and have not achieved, whom you know, and how you relate has repercussions for the success or failure of an enterprise, whether scientific or commercial. Oral history is probably better than any other methodology for documenting these personal dimensions of history. Its vivid descriptions of personalities and events not only make history vital and engaging, but also contribute to an understanding of why circumstances occurred in the manner they did.

Molecular biology and biotechnology are fields with high scientific and commercial stakes. As one might expect, the oral histories reveal the complex interweaving of scientific, business, social, and personal factors shaping these fields. The expectation is that the oral histories will serve as fertile ground for research by present and future scholars interested in any number of different aspects of this rich and fascinating history.

Update, September 2001

In early 2001, the Program in the History of the Biological Sciences and Biotechnology was given great impetus by Genentech's generous pledge of one million dollars to support documentation of the biotechnology industry. At an initial meeting of Genentech and Library personnel in November 2000, it was agreed that the initial phase of the Genentech-supported project in the company's twenty-fifth anniversary year should focus on oral histories

with current and former Genentech employees. Archival collection, on the other hand, was designated as a long-term process because of the greater necessity to gather oral documentation while minds are clear and because of Genentech's present need to retain many corporate documents for legal and other reasons.

On October 15, 2001, The Bancroft Library will celebrate Genentech's twenty-fifth anniversary and acknowledge its generosity to the Program by formally presenting the oral histories of Herbert W. Boyer and Robert A. Swanson, the company's founders. Oral histories are currently in progress with the following individuals presently or formerly at Genentech: David Goeddel, Arthur Levinson, Fred Middleton, Richard Scheller, and Daniel Yansura. Oral histories are also completed or in progress with individuals at Chiron Corporation and Tularik, Inc. The next phase will expand documentation to other biotechnology companies.

Location of the Oral Histories

Copies of the oral histories are available at the Bancroft, UCSF, and UCLA libraries. They also may be purchased at cost through the Regional Oral History Office. Some of the oral histories, with more to come, are available on The Bancroft Library's History of the Biological Sciences and Biotechnology Website: http://www.lib.berkeley.edu/BANC/Biotech/.

Sally Smith Hughes, Ph.D.
Historian of Science

Regional Oral History Office
The Bancroft Library
University of California, Berkeley
October 2001

Program in the History of the Biological Sciences and Biotechnology
Completed Oral Histories

November 2001

Horace A. Barker, *Scientist and Professor of Microbial Biochemistry at Berkeley*, 2001

Paul Berg, Ph.D., *A Stanford Professor's Career in Biochemistry, Science Politics, and the Biotechnology Industry*, 2000

Herbert W. Boyer, Ph.D., *Recombinant DNA Research at UCSF and Commercial Application at Genentech*, 2001

Arthur Kornberg, M.D., *Biochemistry at Stanford, Biotechnology at DNAX*, 1998

Niels Reimers, *Stanford's Office of Technology Licensing and the Cohen/Boyer Cloning Patents*, 1998

William J. Rutter, Ph.D., *The Department of Biochemistry and the Molecular Approach to Biomedicine at the University of California, San Francisco, Volume I*, 1998

Robert A. Swanson, M.S., *Co-Founder, CEO, and Chairman of Genentech, Inc., 1976-1996*, 2001

Oral Histories in Process

Stanley N. Cohen, M.D.
David Goeddel, Ph.D.
Dennis Kleid, Ph.D.
Daniel E. Koshland, Ph.D.
Marian E. Koshland, Ph.D., retrospective
Arthur Levinson, Ph.D.
Fred Middleton
Thomas Perkins
Reorganization of Biology at UC Berkeley
William Rutter, Ph.D., Volume II
Richard Scheller, Ph.D.

INTRODUCTION by Clinton E. Ballou, Ph.D.

Professor H. A. Barker was born on November 29, 1907, in Oakland, California, where he grew up not far from Lake Merritt. At an early age, he was given the nickname "Nook" by family members, who saw a close resemblance to a popular cartoon character with a similar name (1). This nickname stayed with him all his life and was adopted widely by friends and colleagues. Later, in official correspondence, he signed his name "Horace," although on less formal documents he occasionally used "Al," a variant of his middle name. He was introduced to his favorite avocation at an early age, when he went trout fishing on some of the small streams that flowed from the Oakland and Berkeley Hills into San Francisco Bay (2), and this love for nature and the outdoors lasted throughout his life. When he was eleven, the family moved to Palo Alto where his father, Albert C. Barker, was a teacher and school administrator (3). Before their marriage, both of his parents had attended Stanford University, where his future mother, Nettie Hindry, obtained degrees in classical literature and Latin, and it is possible that the name Horace reflected her classical interests.

Barker has noted that "Both my father and mother were very fond of the outdoors and so each summer we spent a month or more, whenever possible, camping in the Sierras, and living a quiet and simple life in close contact with Nature. This resulted in my developing a considerable familiarity with plants and animals, and the physical environment, and perhaps even more important, developing a sense of satisfaction and accomplishment in relatively solitary activities such as fishing, hiking, and exploring new areas; this attitude was easily carried over to scientific work in a laboratory." (3) He also developed an interest in music, played the piano, and, after graduation from high school, spent a year in Germany with his family where he "learned German, read classical German literature, and went to innumerable operas and concerts of every kind." (3) In 1925, he entered Stanford University, obtained an undergraduate degree in physical sciences, and left there in 1933 with a Ph.D. in Chemistry. While at Stanford, he also met and married Margaret D. McDowell, with whom he had three children, Barbara Freide of Piedmont, California, Betsy Mark of Lexington, Massachusetts, and Bob Barker of Camino, California.

In his oral history, it is made clear that the three years following his graduation from Stanford were a formative time in stimulating his interest in microbiology and then concentrating his attention on microbial biochemistry as the focus of his academic career. In 1933, Barker set out on a two-year fellowship to study with C. B. van Niel at the Hopkins Marine Station on Monterey Bay. He was the first postdoctoral student of this young Stanford assistant professor who was to become an icon of microbiology and a magnet that, over several

decades, attracted many scientists with an interest in microbiology to enroll in his famous summer course at the station. Although Barker had developed some interest in biology as an undergraduate, particularly botany, it was at Pacific Grove that he really committed to the subject. He once recalled with awe how he would meet regularly with van Niel for a one-on-one lecture, given entirely without notes, that might run for an hour or more while delving deeply into some current topic. (3)

Barker also credited van Niel with introducing him to the valuable technique of enrichment culture for the isolation of microorganisms capable of effecting almost any desired biochemical reaction. I was a beneficiary of this indoctrination when, in the 1960s, my research on the structure of yeast mannans was stymied for lack of enzymes that could selectively degrade the carbohydrate chains. In frustration, I sought the council of then Professor Barker, who advised me to get some rich dirt, put it in a test tube with an aqueous solution of mannan, and wait until something grew up. Any microorganism that grew must be able to hydrolyze the polysaccharide to give free mannose, a sugar similar to glucose, that the organism could use as an energy source. Success followed success with several different mannans, and we soon had a collection of bacterial strains for the isolation of different mannosidases (enzymes) with which we could take the polysaccharide apart in a selective and stepwise manner.

When his fellowship at the Marine Station ended in 1935, Barker backtracked on the footsteps of his mentor van Niel to study for a year with A. J. Kluyver at the Delft Microbiology Laboratory. It was during this year in Holland that he initiated an investigation that many years later would lead him to the important discovery of vitamin B-12 coenzymes. (3) Also, during this year Barker received an invitation from the University of California at Berkeley to join the Agriculture Experiment Station as an instructor in soil microbiology, an opportunity he attributed to van Niel's intercession. Two other promising young biologists, William Zev Hassid (in plant nutrition) and Michael Doudoroff (in bacteriology), accepted Berkeley appointments during the same era, and later these three were to become closely associated in teaching and research, with results that would bring distinction to the campus.

In his oral history, Barker emphasized events prior to 1950, when he was getting started at Berkeley, and perhaps these years were the most memorable to him because they had such an influence on his later career. It would be unfortunate, however, for anyone reading the history to come away without a more complete picture of the man and his role in science. To bring this picture into focus, it is helpful to list some of the outstanding scientists who as students gained their training with Barker, who worked as visitors in his laboratory, or with whom he had an important association. These include Earl and Thressa Stadtman, Fritz Lipmann, Eugene Kennedy, Joseph Wachsman, Irwin Gunsalus, Arthur Kornberg, Jesse Rabinowitz, Herbert Weissbach, Harry

Hogenkamp, Benjamin Volcani, Roscoe Brady, Gerhard Gottschalk, Bernard Horecker, Ralph Costilow, Robert Switzer, Robert Blakeley, Ching C. Wang, and Ernst Winnacker. (4) Thus, Barker was mentor to a number of young scientists who went on to outstanding careers, and he attracted many distinguished investigators from around the world to his laboratory in Berkeley.

Barker's major research activities dealt with studies on anaerobic fermentation by bacteria. He elucidated a general pathway in bacteria for the formation of methane from carbon dioxide, acetate and methanol, and in so doing he was the first to demonstrate (with Sam Ruben and Martin D. Kamen) the use of the long-lived radioactive isotope carbon-14 "as a tracer in a biological system." (5) Then, using similar techniques, he demonstrated the reductive incorporation of carbon dioxide and of ethanol into short-chain fatty acids and various amino acids. Turning his attention to the bacterial fermentation of amino acids, he uncovered new pathways for their decomposition that, with glutamate as a substrate, involved a novel chain rearrangement. This reaction was found to be dependent on vitamin B-12, which led Barker to the isolation and partial characterization of the coenzyme forms of the vitamin, reported in 1960. A detailed account of these and other studies is given in (3). In 1964, the British chemist Dorothy Hodgkin was awarded the Nobel Prize for her work on vitamin B-12 structure, and many of his colleagues feel that Barker could have shared in the prize. This view was supported recently by Professor J. R. Quayle, F.R.S., who observed that, "Looking back at Barker's overall achievements there is no doubt that they are world-class, at the Nobel level. He entered the field of bacterial fermentations when mixed cultures were the order of the day. He and [Robert] Hungate developed pure culture isolation techniques to the point that many people came to Berkeley to find out how. Barker's intuition and meticulous analytical approach reduced complex fermentations into a series of intellectually elegant equations and carbon balances. In this he could be matched by few. He entered the methanogenesis field in its prehistoric state and, again, provided cultures and a chemical rationale that guided workers in the field for years to come." (6)

In unpublished notes prepared by Barker in 1969 (5), the following two sentences appear. "Before coming to Berkeley in September 1936, I had investigated the biological formation of methane from ethanol, acetate and butyrate and had obtained evidence for the theory of C. B. van Niel that methane is formed by reduction of carbon dioxide. In the fermentation of ethanol in the presence of calcium carbonate by enrichment cultures of methane bacteria, I found that one mole of carbonate was reduced to methane for each two moles of ethanol oxidized to acetate." Here we see stated the topic that would occupy Barker for much of his career as he sought to define the biochemical mechanisms involved in such a seemingly simple transformation. Although he was taken on several side journeys along the way, finding the mechanisms and pathways in bacteria by which carbon dioxide was utilized as an oxidant,

by which methane was produced, and by which various other products resulted from the fermentation of ethanol and amino acids, would consume most of his energy for the years to come.

Also contained in one of these sentences is a hint of the special characteristic that defined Barker's approach to science. He was careful in designing his experiments and meticulous in accounting for the stoichiometry of the reactants and products in any investigation. Because of this practice, he was led to insights that might have eluded the less attentive investigator. Thus, when he wrote, "I found that one mole of carbonate was reduced to methane for each two moles of ethanol oxidized to acetate," (5) one could rely on this as being significant and near to the truth. Sometimes, however, he appeared to carry this concern for numbers and accountability to an extreme. One cold winter day in December, we were taking a trip together by car to fish for steelhead on the Eel River, and Barker was driving. He decided to stop for gas, and I was surprised when he withdrew a small black notebook from the glove compartment and recorded the date and the exact mileage on the speedometer, along with the amount of gas purchased and the cost. I also saw that it was a well-used book that contained page after page with columns of similar figures, and I have long pondered his attention to such detail. I should add that this fishing trip also revealed another characteristic of Barker, namely the persistent determination with which he approached a problem. On this day, the problem was to catch a fish, and steelhead are not the easiest quarry to pursue successfully. After about eight hours of fruitless casting on that cold and blustery day, I had given up, but Barker continued on until, when the rest of us had reeled in our lines and were ready to leave, he set the hook and eventually landed a seven-pound beauty. Mission accomplished.

In his daily approach to science, Barker was somewhat detached and completely unperturbable. He did not concern himself with the real or imagined threat from competitors that motivates many scientists. Perhaps he sought consciously to avoid research projects where the competition was extreme. On one occasion, however, when his lab was zeroing in on the B-12 coenzymes, he was faced with such a situation. To learn first-hand how he acted at the time, I asked Herbert Weissbach, a visiting scientist from the NIH and Barker's main collaborator on this project from 1958-60, to share his experience. He wrote (7) "My year with Barker was truly unforgettable. I would not say he was 'laid-back' but he certainly had complete control of his emotions...the day we showed the unknown cofactor for the conversion of glutamate to beta methyl aspartate was a derivative of vitamin B-12. I had taken this orange solution which had a spectrum that was not similar to anything known and, after exposing it to light, the color changed and the new spectrum was that of hydroxy B-12. This must have been in July or August, on the day before Nook was to leave for his vacation home. I came running into his office with what I thought was exciting news and he said that the results were very nice. I suggested that we discuss

what experiments to do and put together a manuscript as quickly as possible. He just continued what he was doing and said it could wait until after he came back from vacation. Having come from the NIH, where in a similar situation the manuscript would have been written that day, I was shocked to say the least. I remember meeting Esmond Snell in the hall and telling him about Nook's reaction, which to my amazement didn't surprise him at all."

In the 1940s, Barker, Doudoroff, and Hassid were located in neighboring labs on the third floor of the Life Sciences Building, in what today might be considered minimal accommodations. Fortunately for science, however, this close association brought them to collaborate on a project that was concerned with the biosynthesis of sucrose, ordinary table sugar. It was the bacteriologist Doudoroff who first observed the phosphate-dependent cleavage of this disaccharide by the bacterium *Pseudomonas saccharophila*, which produced glucose 1-phosphate and fructose. The enzyme that catalyzed this reaction was purified and named sucrose phosphorylase. Later, Doudoroff and Hassid, a carbohydrate chemist, showed that the reaction could be reversed to form a nonreducing sugar that appeared to be sucrose. At this time, Barker joined the project, probably to bring his chemical training to bear in proving the identity of the putative sucrose. When published, this research led to an amusing incident, as described in Hassid's obituary. (8) "The enzymatic synthesis of sucrose resulted in some publicity that came to the attention of officials of the Coca-Cola company, who were having difficulty obtaining sucrose because of wartime rationing. The company sent a representative to Berkeley to ascertain whether commercial quantities of sucrose could be made by the enzymatic method. Hassid and his associates were away on vacation at the time, so the Coca-Cola emissary discussed the problem with (then Dean) Professor Hoagland and reported that his company was prepared to provide $500,000 for research on this enzyme if a commercial process of sucrose synthesis seemed feasible. Unfortunately, Professor Hoagland was pessimistic about the possibility of sweetening Coca-Cola by this method, and so further support of research on sucrose phosphorylase was left to the University and the U.S. Public Health Service." Considering the magnitude of this proposal, one can only wonder about the outcome had Dean Hoagland left the matter for Barker, Doudoroff and Hassid to negotiate with the Coca-Cola Company.

Barker has described his role in facilitating graduate study in biochemistry at Berkeley by helping to start the Comparative Biochemistry Group Major. In general, group majors are a device for bypassing formalized departmental regulations in order to create interdepartmental programs that accommodate to the special needs of certain students and faculty. A perceived weakness, according to some, is that the relaxed administration of such programs can lead to less rigor in admission requirements and to poor supervision of the students. Regardless, as Barker noted (3), "From 1936 to 1948 my students obtained advanced degrees in the graduate curricula of Bacteriology,

Microbiology, or Agricultural Chemistry. The Biochemistry Department at Berkeley during that period was part of the Medical School; graduate degrees in biochemistry were not available to students studying with other faculty members. Since many students in other departments were doing research on biochemical problems and wished to be recognized as biochemists, there was considerable interest among both students and faculty in setting up an academic mechanism for giving degrees in biochemistry outside of the Biochemistry Department." Thus, in 1948, Barker helped to establish a Ph.D. curriculum in comparative biochemistry, which he administered until his retirement in 1975, and during which time about seventy-five students were awarded degrees. As he notes, the later performances of students such as Elizabeth Neufeld, Paul Srere, and Earl Stadtman suggest that quality did not suffer during his tenure in the program. In 1980, there were eleven such group majors in the biological sciences, the one in comparative biochemistry including faculty from biochemistry, cell physiology, chemical biodynamics, chemistry, entomology, forestry, immunology, Lawrence Berkeley Laboratory, molecular biology, nutritional science, physiology-anatomy, public health, and UCSF biochemistry and biophysics. (9) Clearly, the group majors have played a significant role on the Berkeley campus.

In his formal teaching, Barker concentrated his efforts mainly in two areas. One was an undergraduate laboratory course that he inherited upon his appointment in 1937 from C. B. Lipman. This course initially dealt with soil microbiology, but over the years it evolved into a plant biochemistry laboratory. When Barker later joined the biochemistry department in the College of Letters and Science, this course served as a model for the Biochemistry 102L Laboratory that for many years was offered by the department for nonmajor students. Barker also developed a graduate lecture course in microbial metabolism in collaboration with Doudoroff, and taught the course in the Department of Bacteriology with Doudoroff, Roger Stanier, and Edward Adelberg. In all of his teaching, Barker was straightforward, methodical, and well-organized, and he never indulged in showmanship or intentional humor, nor did he consciously aim to be entertaining. He gave his teaching the same serious consideration he gave to his research, and my observations suggest that the students respected him for this.

During his career at Berkeley, Barker appears to have been a reluctant administrator, although he did chair the small departments of plant nutrition (1949-50) and plant biochemistry (1950-53), and he served two years as chairman of the biochemistry department (1962-64) at the difficult time when it was preparing to move into new quarters and sever connections with the Virus Laboratory. Years earlier, when the Biochemistry and Virus Laboratory was completed in 1951, Barker had moved onto the third floor along with several other members of the newly formed Department of Agricultural Biochemistry. He has noted (3) that "Although the laboratories were an improvement over those we had previously occupied, the administrative arrangements in the building

were difficult for several years because of an almost constant struggle over authority and space." This is a muted reference to his relationship with Wendell Stanley, who was recruited in 1948 to head up the new biochemistry department in the College of Letters and Science and to serve as director of the Virus Laboratory. (10) Fortunately for all concerned, this source of conflict was eliminated in 1964 when most of the biochemistry faculty moved to a new building at the west end of the campus.

In one of the experiments carried out by Barker in Delft, he observed the accumulation of large amounts of n-caproic acid during the anaerobic fermentation of ethanol by a bacterium he had isolated by enrichment culture, and Kluyver brought this result to the attention of a local chemical manufacturer for possible exploitation. Barker reports, however, "So far as I know nothing ever came of this. Nevertheless, the company provided me with a small retainer that made it possible, the following year, to start construction of a cabin in the mountains of California we still use each summer." (3) This cabin, built in 1937 at Silver Lake near Mount Lassen, is well-known to many of Barker's friends and colleagues who have been lucky enough to spend a few days there with him and his wife Margaret during the wonderful summer days that visit the northern Sierras. They loved books and often read to each other at home or at Silver Lake. The lake was also a place for playing the word game Scrabble, and my wife and I often competed with them in the evening before a roaring fire in their cabin. Margaret always seemed to command the broadest and most esoteric vocabulary, but her husband was a superior tactician when it came to utilizing the available word combinations.

Visitors to the cabin were always taken on long hikes to the numerous upper lakes in the so-called wilderness area, and along the way they were introduced to every plant, insect, and mammal by the official Latin names and were given a description of their most interesting characteristics. On my first visit to Silver Lake about 1970, I asked Barker if he had a map I could use while hiking by myself. He pulled a sheet of yellow paper from a drawer and drew from memory all of the trails, cliffs, ponds, and lakes for the surrounding five mile area, and on it he indicated the best places to fish on each lake. My personal map was done with such accuracy that I still use it today, some thirty years later. The only thing it lacks is the location of the several small "secret lakes" that Barker and his son Bob stocked early each spring with fingerlings they caught and transplanted from the larger surrounding lakes. After the fish matured for a year or two in these food-rich lakes, the Barkers returned to harvest the reward. This and other pleasant activities at the lake served to draw Barker back to his cabin each summer for over sixty years.

After his retirement in 1975, mandated by the age limit then enforced at the University of California, Barker maintained a regular schedule of attendance on campus. Although he endured a heart attack

while hiking at Silver Lake in 1987 and had undergone bypass surgery, he recovered quickly and his health remained good. For many of these later years, he faithfully attended his wife, Margaret, during a prolonged confinement that preceded her death in 1995. Shortly after his ninety-third birthday, Barker suffered a brief illness and died from heart failure at his home in Berkeley on December 24, 2000. (11)

In ending this review, it is fitting to recall the many honors that accrued to Barker during his career (12), which include the Sugar Research Award in 1945, election to the National Academy of Sciences in 1953, the Carl Neuberg Medal in 1959, the Borden Award in 1962, the California Scientist of the Year Award in 1965, the F. G. Hopkins Medal of the British Biochemical Society in 1967, the National Medal of Science in 1968 presented to him at the White House by President Lyndon Johnson, and the University of California Berkeley Citation in 1975. In 1988, the Biochemistry Building was renamed Barker Hall and Barker's portrait was hung in the lobby where it will long bring enduring recognition to this remarkable man.

Clinton E. Ballou, Ph.D.
Professor Emeritus, Biochemistry

February, 2001
University of California, Berkeley

References

1. Barker's son, Bob, suggested (December 24, 2000) that "Nook" is derived from "Snookums." According to *The World Encyclopedia of Comics* (Maurice Horn, ed., Chelsea House Publishers, 1976), Snookums is the name of the infant character in the comic strip "The Newlyweds" by George McManus, published as a newspaper Sunday feature from 1904-1918.

2. Personal comment by H. A. Barker.

3. H. A. Barker, "Explorations of Bacterial Metabolism," *Annual Reviews of Biochemistry* 1978, 47:1-33.

4. H. A. Barker curriculum vitae and bibliography (1978?).

5. H. A. Barker, "Notes on the history of biochemistry at Berkeley," December 15, 1969.

6. J. R. Quayle, personal communication, January 26, 2001.

7. H. Weissbach, personal communication, January 5, 2001.

8. C. E. Ballou and H. A. Barker, "Willaim Zev Hassid (1899-1974) A Biographical Memoir," *Proceedings of the National Academy of Sciences* 1979, 50:197-230.

9. University of California, Berkeley, General Catalog 1980.

10. A. N. H. Creager, "Wendell Stanley's Dream of a Free-standing Biochemistry Department at the University of California, Berkeley," *Journal of the History of Biology* 1996, 29:331-360.

11. H. A. Barker obituary, *San Francisco Chronicle*, January 5, 2001.

12. *American Men and Women of Science*, 15th edition, R. R. Bowker Co., 1982.

INTERVIEW HISTORY--Horace A. Barker

Horace Barker was interviewed for the Bancroft Library's Program in the History of the Biosciences and Biotechnology as part of its effort to document basic science contributions to biomedicine and the biotechnology industry. From the 1930s on, Barker pursued a basic biochemical approach to microbiology focused on natural history and metabolism of soil bacteria at a time when many others in the field were studying microorganisms as pathogens.

We are grateful to Dr. Barker for persevering at age ninety-one and despite ill health through three interview sessions in which he provided the outline of his professional achievements, particularly those early in his career. Highlights of his story are his two summers and one fellowship year in the 1930s with the eminent Dutch microbiologist, Cornelius van Niel at Stanford's Hopkins Marine Station; his postdoctoral fellowship under A. J. Kluyver at the Delft Laboratory of Microbiology in Holland, and his long service, 1936-1975, on the Berkeley faculty, first in the University of California Agricultural Experiment Station and later in the department of biochemistry.

Of particular interest is Barker's work beginning in the late 1930s with the use of artificial radioisotopes produced by Ernest Lawrence's 60-inch cyclotron in Crocker Radiation Laboratory on the Berkeley campus. Barker tells in the oral history of his collaboration with the physical scientists Martin Kamen, Sam Ruben, and Zev Hassid in some of the earliest work anywhere using artificial radioisotopes in biological tracer experiments. His account is a useful extension of the series of oral histories in the Bancroft Library on medical physics at Berkeley, which include documentation of the earliest synthesis and application of artificial radioisotopes in biology.

We would like to have heard more about Barker's many other accomplishments, unfortunately only partially recounted here, in instilling comparative microbial biochemistry at UC Berkeley. His renown rests on "a lifetime record of stellar achievements" in basic science, as the Nobel laureate Arthur Kornberg commented.[1] Barker performed pioneering work on elucidating metabolic pathways in soil bacteria, including his work on vitamin B_{12} for which UC held a patent and which became of considerable interest to the pharmaceutical firms Merck and Squibb. Thus Barker's work presents an early example of the commercial potential of basic biological research well before the recombinant DNA revolution of the 1970s and the growth of the modern

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[1] Arthur Kornberg, *For the Love of Enzymes: The Odyssey of a Biochemist*, Cambridge: Harvard University press, 1989, p. 172.

biotechnology industry. Fortunately, Barker some years ago carefully arranged and then donated to the Bancroft Library more than eleven cartons of his correspondence, laboratory notebooks, and assorted photographs documenting his scientific contributions. His lengthy review of his own scientific career, published in the *Annual Review of Biochemistry*, is available in the appendix of this oral history, along with his curriculum vitae, bibliography, and other relevant documents. These documents help to fill in what Dr. Barker left out of the oral history, but fail to provide the social context and personal dimensions of his activities.

Also missing in the oral history is documentation of Barker's role in helping to recruit many of the biochemists and molecular biologists who were to make UC Berkeley a center of the biochemical and molecular approach in the life sciences. Barker does however mention the Program in Comparative Biochemistry, an interdepartmental group which provided a broad forum for faculty and graduate students interested in biochemistry. He also hints at, but falls short of elaborating on, the animosity between Wendell Stanley and his group in the Virus Lab, and the biochemists whom Stanley had hoped to unite in Berkeley's first department of biochemistry, founded in 1950. Barker was in fact one of Stanley's prime opponents, objecting among other things to Stanley's attempt to focus biochemistry on viral research. As a result of these and other professional and personal tensions, Stanley resigned in 1953 as chairman of biochemistry. In the end, three separate departments emerged from the group that Stanley had striven unsuccessfully to unite: biochemistry, virology, and molecular biology. Thus Stanley's vision of a unified biochemical and molecular enterprise on campus was only realized in the 1980s and nineties when these fields were organized under a new Department of Molecular and Cell Biology. For full historical treatment of these developments, the reader is referred to an article by Angela Creager[2] and an oral history in The Bancroft Library series on the reorganization of biology at Berkeley.

Oral History Process

Three interviews were conducted with Dr. Barker between December 21, 1998, and January 14, 1999. The first was conducted in Dr. Barker's office in Barker Hall on the Berkeley campus, with biochemistry colleagues Clinton Ballou and Edward Penhoet in attendance. We are grateful to Dr. Penhoet for instigating the idea for and funding interviews with his mentor Dr. Barker. The later interviews were conducted one to one, in Barker's modest home in Berkeley where he lived alone after the death of his wife Margaret in 1995. Soft spoken and

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[2]Angela N. H. Creager, "Wendell Stanley's Dream of a Free-standing Biochemistry Department at the University of California, Berkeley," *Journal of the History of Biology* 1996, 29:331-360.

reserved, Dr. Barker answered to the best of his ability but had trouble remembering details of recent history. We thank Dr. Barker's daughter, Barbara Friede, and Louise Taylor, long a friend of the Barker family, for reviewing the transcripts. They made only very occasional changes and additions.

We are particularly indebted to Clinton E. Ballou, Ph.D., for extending the information and accuracy of the oral history. He painstakingly reviewed the transcripts, provided biographical information for people mentioned by Dr. Barker, corrected spelling of proper names, and so on. In doing so, Dr. Ballou pulled on his long association with Barker as colleague and friend in the Berkeley biochemistry department. As emeritus professors, they shared an office in Barker Hall, the location of the first interview. In addition, Dr. Ballou carefully researched and wrote the introduction to this volume. Although there is no equivalent to an oral history of Dr. Barker recorded in his prime, thanks to Dr. Ballou's contributions, for which we are truly grateful, the present volume is the next best thing. Dr. Ballou's introduction describes Barker's scientific contributions and fills in details which Dr. Barker was unable to provide about the postwar development of biochemistry on the Berkeley campus. As a result, we believe that by using the combined resources of the introductory material, the interviews, and the appendix contents, the reader will obtain a good sense of Dr. Barker and his science. For the serious researcher, this oral history will provide a useful platform for further research.

This oral history reflects the contributions and working environment of a remarkable scientist who is widely respected and did much to advance the field of comparative microbiology, particularly in the area of bacterial metabolism.

Dr. Barker died quietly at home on December 28, 2000, before the oral history volume was completed.

The Regional Oral History Office was established in 1954 to augment through tape-recorded memoirs the Library's materials on the history of California and the West. Copies of all interviews are available for research use in The Bancroft Library and in the UCLA Department of Special Collections. The office is under the direction of Richard Cándida Smith, Director, and the administrative direction of Charles B. Faulhaber, James D. Hart Director of The Bancroft Library, University of California, Berkeley.

Sally Smith Hughes, Ph.D,
Historian of Science and Project Director

July 2001
Regional Oral History Office
The Bancroft Library
University of California, Berkeley

Regional Oral History Office
Room 486 The Bancroft Library

University of California
Berkeley, California 94720

BIOGRAPHICAL INFORMATION

(Please write clearly. Use black ink.)

Your full name HORACE ALBERT BARKER

Date of birth November 29, 1907 Birthplace Oakland, CA

Father's full name ALBERT CHARLES BARKER

Occupation Superintendent of Schools - PALO ALTO Birthplace MAINE

Mother's full name Nettie HINDRY BARKER

Occupation ACCOUNTANT Birthplace Denver, Co.

Your spouse MARGARET McDOWELL BARKER

Occupation Housewife Birthplace PALO ALTO, CA

Your children BARBARA, ELIZABETH, ROBERT

Where did you grow up? PALO ALTO, CA

Present community Berkeley, CA

Education B.S. STANFORD, Ph.D. STANFORD

Occupation(s) Professor of Biochemistry U.C. Berkeley

Areas of expertise

Other interests or activities FAMILY, Fishing, Hiking

Organizations in which you are active

INTERVIEW WITH HORACE BARKER

I HIGHER EDUCATION, 1926-1936

[Interview 1: December 21, 1998][1] ##[2]

Stanford University, 1926-1933

Research Assistant with C.V. Taylor, 1930-1931

Hughes: Well, do you want to start with your undergraduate years at Stanford [1925-1929]? I know from your review article[3] that you had a hard time deciding whether it was going to be the humanities or the sciences that you were going to major in.

Barker: Well, my family--my brother in particular--had been in the humanities. He ended up as an English professor. He was a Rhodes scholar and he was at Oxford for several years where I visited him briefly. He was at Stanford after that but then he ended up in the eastern United States for most of his career.

Hughes: Do you remember how you decided on the sciences rather than the humanities?

Barker: Well, I got started in biology; that's really why I got into science. When I was an undergraduate I got invited by a professor in the biology department, whose name escapes me at the moment.

Hughes: Taylor?

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[1] Also present: Edward E. Penhoet and Clinton E. Ballou.

[2] ## This symbol indicates that a tape or tape segment has begun or ended. A guide to the tapes follows the transcript.

[3] H. A. Barker. "Explorations of bacterial metabolism." *Annual Review of Biochemistry* 1978, 47:1-33.

Barker: C. V. Taylor. I worked with him somewhat and then he was invited to the University of Chicago and invited me to come along as a research assistant, so I was there for a year [1930-31].

Hughes: Was it unusual for an undergraduate to go off with a professor?

Barker: Let's see, was I an undergraduate at the time? I got my A.B. in 1929, I believe.

Hughes: Yes, you're right.

Barker: So I was a young graduate student. January term I took a course from Taylor. And it was a very small course; I think there were only about half-a-dozen students in it. Evidently I clicked in some way with him and so he invited me to come to Chicago. So I learned about the stockyards. [laughs]

Hughes: Did that experience get you interested in microbes? Taylor, as I remember, was a protozoologist.

Barker: Yes, he was a protozoologist. Well, the thing that really got me started in microbiology was a course I took with [Cornelius] van Niel one summer at Pacific Grove [California]. Van Niel was a very prominent man in the area of microbiology. He discovered most synthetic bacteria and a variety of other things. He left Holland shortly after he got his Ph.D. and came to Pacific Grove, and I was one of his first students.

Thesis in the Chemistry Department

Penhoet: Did you do your thesis work at Pacific Grove?

Barker: No, I did my thesis in chemistry at Stanford, with [James W.] McBain.

Hughes: And what was the subject?

Barker: Well, let's see, it was a long time ago.

Hughes: Was it microbial?

Barker: No, I worked on egg albumen--denaturation of egg albumen.

Penhoet: Oh, so it was biochemistry.

Barker: It was biochemistry.

Penhoet: In the chemistry department at Stanford. That's interesting.

Barker: Well, see, [Murray] Luck was a biochemist. He was in the chemistry department for many years.[4] He wasn't a very good teacher; his main activities were in the *Annual Review* [*of Biochemistry*], I think.

Hughes: So it sounds as though from a relatively early age you were interested in the biochemical aspects of microorganisms, is that right?

Barker: Right.

Hughes: How did you get on that track?

Barker: Well, I think it was probably initially from my contacts with C. V. Taylor. He was later the chairman of the biology department at Stanford for a number of years.

Hughes: But he had a biochemical interest?

Barker: Well, he had a biochemical interest, yes, but he was really more of a biologist than he was biochemist.

Penhoet: Going further back, Nook, undergraduates at Stanford were encouraged to get involved in research? You told me, for example, that several summers you took a car with another undergraduate and drove all over the southwest collecting plants.

Early Interest in Botany

Barker: But that was unconnected with any push by anybody on the faculty. At Stanford in the early days I got interested in botany. I took a course in systematic botany with [L. R.] Abrams, who was in charge of the herbarium at Stanford. It was a course in which you took field trips and went out to various places around the Bay Area. And once we went up to Yosemite, I remember.

We collected various things and brought them back and classified them. It was a rather traditional taxonomy course.

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[4] Luck was a biochemist in the Department of Chemistry; in 1959, Stanford created its first department of biochemistry, with Arthur Kornberg as chairman. For a history of the latter department, see the oral history with Kornberg in The Bancroft Library Series.

But I got interested since I had been going into the mountains for many years and I began collecting things on my own. I had a substantial collection which I contributed, ultimately, to the herbarium of Stanford. I don't know what's happened to it since then, but it was moved to San Francisco at one stage, to the Academy of Sciences.

Penhoet: So your interest in nature came from your own family experience.

Barker: Well, it came from the mountains, really. Yes, at some stage I started collecting plants in the mountains.

Penhoet: This was before you were even an undergraduate--when you were a child?

Barker: Oh, well, I'm sure to some degree it had an influence, but I didn't really begin collecting in any formal way until I was a student.

Hughes: This was something that you did on your own. It wasn't in connection with any class.

Barker: Well, I took a course in systematic botany at Stanford and I think that stimulated it. We took a number of field trips and we went to Yosemite in the fall once. We went to various places to collect so later on I took this more seriously than I did on my own. [laughs]

Penhoet: Was that typical of the education at Stanford at the time, that students got involved in field trips and doing things, rather than just reading out of books?

Barker: Oh, yes, I think so. At least as they began to get into some research area, or potential research area.

Ballou: How many students were there at Stanford at that time, do you have a recollection? It must not have been a very large university, was it?

Barker: No, it wasn't really large. I don't know how many, but it was much, much smaller than Berkeley in those days.

Hughes: There was a lot of contact between students and faculty?

Barker: Yes. There was quite a lot of contact with faculty.

Cornelius van Niel and the Hopkins Marine Station, Pacific Grove, California, 1931-1935

Van Niel as a Personality

Hughes: Well, let's go back to van Niel and the Hopkins Marine Station.

Barker: All right.

Hughes: Could you say something about him as a personality?

Barker: Oh, he was a very impressive guy. [laughs] He was very, very outgoing, and he had an impressive way of speaking.

Hughes: Was he fluent in English?

Barker: Very fluent in English, oh, yes. In Holland, you know, they learn German, French and English, in grammar school, I guess. Yes, he spoke quite fluently. He had a slight Dutch accent. [laughs] And he was a great teacher.

Hughes: What was his approach to microbiology?

Barker: Well, he had a great interest in the biochemical aspects.

Hughes: You were there [at Hopkins Marine Station] two summers [1930, 1931], and then also for a National Research Council Fellowship [1933-35].

Barker: Yes, I was.

Work with J. P. Baumberger, Summer 1931

Hughes: Did van Niel have projects for you to do, or did you come with projects in mind?

Barker: Well, let's see, I think the first time I was there was not with him; it was connected with a professor whose name escapes me at the moment, who was in the physiology department at Stanford. Do you have any names there?

Hughes: Yes, there's a J. [James] P. [Percy] Baumberger.

Barker: Yes, Baumberger. I think I was originally an assistant in some of his classes. I didn't get acquainted with van Niel until later.

Hughes: Not until your fellowship?

Barker: Yes.

Hughes: How did a day go? Were you working shoulder to shoulder, so to speak, or did everybody have more or less independent projects?

Barker: Well, the Hopkins Marine Station is a very small place, and so at that time there were two sets of activity. There were some people who were interested in invertebrates. They would get up early in the morning and collect when the tide was low, that sort of thing. I did a little of this because we did it in connection with classes.

Working with van Niel

Barker: But then van Niel came and he had no direct connection, I think, to this sort of thing. He had been trained in Delft in Holland by A. J. Kluyver, which is sort of a famous name in general microbiology. Van Niel had a very impressive personality, as a teacher and person. [laughs] He had a manner of talking that really was highly impressive. He had a big effect on people, I think, in personality.

Penhoet: The summers that you went to work in his lab, were there a lot of people, or just a few?

Barker: Oh, just a few.

Penhoet: A handful.

Barker: Yes, I was perhaps his first student at that stage. I began working with him. In those days, one had very close contacts with the professors.

Penhoet: So you really worked with him all day, every day, when you were there for the summers?

Barker: Yes. Sometimes we did.

Van Niel's Microbiology Course

Hughes: Was his course hands-on?

Barker: Oh, yes. Well, I think he was probably the most famous teacher in microbiology of his age.

Hughes: Famous people have taken his course.

Barker: I was one of his first students. He used to give me lectures on alcoholic fermentation, for example. He would spend three hours [laughs] lecturing me, and without any notes. I know he studied very hard at night to prepare his lectures.

Ballou: Just for you?

Barker: Well, just for me, to begin with, yes. [laughter]

Penhoet: So he gave you a three-hour lecture just by talking one on one?

Barker: Yes, that's right.

Penhoet: Oh, fantastic!

Hughes: My understanding is that there was never more than a handful of students for the course each summer.

Barker: Well, he was never able to handle more than perhaps ten at the outside, I would guess. But he got people who later became quite well known.

Hughes: My understanding, too, is that it wasn't just the microorganisms themselves, it was also a whole technology that he was teaching, enrichment culture, for example.

Barker: Yes.

Hughes: How widely known was enrichment culture?

Barker: Well, that was a technique which was developed mainly in Holland, well, mainly by [Martinus] Beijerinck. He was the original microbiologist at the University of Delft, starting maybe in the 1890s sometime. And he lived until sometime in the early twenties.[5] I think van Niel actually took some courses from him. But the one van Niel was most closely connected with

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[5] Beijerinck died in 1931.

intellectually and spiritually was A. J. Kluyver. Van Niel was his successor.

Hughes: Wasn't Kluyver a student of Beijerinck's?

Barker: Yes.

Hughes: And van Niel was a student of Kluyver.

Rockefeller Foundation Fellow, Microbiology Laboratory, Polytechnical School[6], Delft, 1935-1936

Physical Layout and Operation of the Microbiology Laboratory

Barker: All this induced me after I got my Ph.D. to spend a year in Holland.

Hughes: Tell me about that, because the Delft school is famous.

Barker: Kluyver lived in a big house on the canal, and this house was directly connected with the laboratory.

Hughes: And Beijerinck had lived there before him?

Barker: I think so, yes. The apartment was right on the canal. You could look out the window and see the canal boats sailing past.

Hughes: What were the laboratories like? How were they equipped?

Barker: Oh, they were very well equipped for those days. And there were various assistants--people that were paid to take care of specialized equipment, to keep track of things and so on. They had a very big collection of microorganisms dating from Beijerinck's time.

Hughes: So they had a series of cultures that were being maintained?

Barker: Oh, yes. Kluyver always had an assistant. It was really a full-time job for somebody who had usually gotten a Ph.D. in the lab or was getting a Ph.D. in the lab. Van Niel had been this at one

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[6] The name in Dutch is Technische Hoogeschool. The literal English translation "technical high school" more correctly translates as polytechnical school or college.

time, and when I was there it was a man by the name of Holdgers who later on became a professor of microbiology in Amsterdam.

Hughes: Once again, this was a metabolic approach, was is not?

Barker: Yes. There was no medical application involved.

Hughes: Was the Delft school a technical school?

Barker: Yes.

Hughes: What difference did that make to the type of research that went on there?

Barker: Well, I don't know that it made any difference, because I had virtually no contacts [with the school]. Except, I remember I was interested in learning something about micro-chemistry--chemical tests on small amounts of things. And Kluyver arranged for me to take sort of a private course with a professor in the chemistry department there, in techniques.

Hughes: At the university?

Barker: At the university there.

Hughes: How big a group was it at the Delft school?

Barker: Oh, well, let's see, I don't know about the university in general, because I had virtually no contact with it, but the microbiology lab had maybe six or eight or ten people doing Ph.D.s. And then Kluyver also taught a course--I think it was two lectures a week--for undergraduate students. That was the only time we saw undergraduates.

Hughes: So it was considered the department of microbiology for the university?

Barker: Yes. It was part of the university.

Hughes: Were you unusual in being a foreign student?

Barker: Well, they had had a succession of foreign students there who had become quite well known.

Hughes: Had you already learned enrichment culture?

Barker: Yes, from van Niel.

Research on Methane-producing Bacteria

Hughes: What did you do that year?

Barker: Well, I became interested in methane-producing bacteria. Holland was a good place to study them because the canal is there. [laughs] And [other] wet places.

Hughes: Is that one reason you chose Delft?

Barker: Oh, no, I went there because van Niel had been there and recommended Kluyver and so on as being a very fine place to be for microbiology.

Hughes: Well, why study methane bacteria particularly?

Barker: Well, I got interested in methane bacteria at Pacific Grove, before I went to Holland. We used to go out occasionally with van Niel on collecting trips around the Monterey area and bring back samples of mud from this, that, and the other place and set up enrichment cultures--cultures in which you'd get methane bacteria. You need an anaerobic environment, so you had some sort of container or bottle which you filled more or less completely with some mud in the bottom, and then some normal nutrients for living organisms. You really want organic compounds--things like acetate or ethyl alcohol, succinic acid--almost anything of that sort, a single compound. You let it sit awhile; pretty soon it begins to bubble, and bubble, and bubble, and it gets more active and pretty soon you have a pretty good enrichment culture.

Ballou: So how do you determine that you have methane-producing bacteria?

Barker: Well, you'd collect some of the gas that's coming off.

Ballou: And light it with a match? [laughs]

Barker: And do a gas analysis on that. We had some sort of system in Pacific Grove for analyzing gas.

Penhoet: So even then it was a little bit of an outgrowth of your interest in collecting things in nature.

Barker: Oh, yes.

Penhoet: But now moving into biochemical analysis of these things in nature.

Barker: Right. Before this I collected plants.

The Enrichment Culture Technique

Hughes: Would it be obvious how one should enrich the culture when you were trying to favor one culture over another?

Barker: Beijerinck was the one who originated the enrichment culture technique. He pushed it very hard. So you'd take a compound and put it in a particular environment. If you wanted an anaerobe, you filled up the bottle with mud and put in the compound and then you waited until something happened. And pretty soon things would begin bubbling, and you knew you had an organism that used this compound and that it probably wasn't isolated, and you could find out more specifically what it did. In many cases it would be a combination of organisms. For example, if you had alcohol as a substrate, it usually would get oxidized to acetate, and then methane bacteria would work on the acetate. There's usually some mud in the bottom, and you'd shake the culture a little and a lot of bubbles would come out.

II BIOCHEMIST AT BERKELEY, 1936-1975

Faculty Member and Soil Microbiologist, UC Berkeley Agriculture Experiment Station, 1936-1950[7]

Penhoet: We were talking about the medical school being separate from the rest of the activities on the [Berkeley] campus.

Barker: Well, originally biochemistry was part of the medical school, and bacteriology also, I believe, under Karl [Friedrich] Meyer.

Hughes: But biochemistry was also located here?

Barker: In Life Sciences [Building]. Biochemistry was on the ground floor and bacteriology was on the third or fourth floor, I think it was at the time.

First Position at Berkeley

Penhoet: How is it that you were in the plant group[8] although your work had always been with microbial systems?

Barker: Well, I was in Holland when I got an invitation to come here. [pause] Well, I'm not really quite sure why they invited me. I suppose they had an opening at that time.

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[7] A portion from the third interview session is included in this section.

[8] From 1936 to 1969, Dr. Barker was on the faculty of the Agricultural Experiment Station, UC Berkeley. In 1949-50, he was chairman of Berkeley's Department of Plant Nutrition. For subsequent appointments and departmental name changes, see his "Biography for Academic Personnel" at the back of this volume.

Penhoet: So it was just an accident of history that the plant group had an opening?

Barker: Well, I don't know whether it was an accident or there was something of more rationality in it, but at any rate I'd never really had any connections with agriculture before. At Stanford I was in the chemistry department, and then I was in Pacific Grove with van Niel for a time. And then I was in Holland. But when I came [to Berkeley], for a number of years the course I taught was soil microbiology.

Penhoet: I see. So that was the connection to agriculture.

Barker: Yes, all the agriculture students at that time had to take a course in microbiology. And I became the one who taught them there in the lab of the Life Sciences Building.

Hughes: Well, that makes sense, with your background, that you would be invited to teach soil microbiology. You were appointed Junior Soil Microbiologist as well as Instructor [1936-1940].

Early Faculty Members in the Berkeley Agriculture Program

Barker: I received an invitation to come to Berkeley I suppose through van Niel's influence. Professor [Dennis R.] Hoagland was looking for somebody to teach soil microbiology to the students of agriculture here.

Hughes: Tell me about Hoagland.

Barker: Hoagland was a plant physiologist, well known here. He was interested primarily in nutrition of higher plants.

Hughes: Was Hoagland doing mainly applied research?

Barker: Well, he did some applied research, but he was interested basically in finding out what elements were required for the growth of plants--usually in water culture--and then seeing what quantities were involved and that sort of thing.

Hughes: Were you the only soil microbiologist?

Barker: Well, before me the person who had taught soil microbiology for a number of years was C. B. Lipman, who was dean of the graduate school. He had a great interest in soil microbiology, so he'd come over two or three afternoons a week and teach a course in a

rather informal way and then go back to his more official office and carry out [laughs] the higher activities of the university. Yes, he was quite a character.

Hughes: Why do you say that?

Barker: Well, I think that Professor Hoagland had a very broad view of research and what its implications were and what it was good for and so on. He was quite supportive. After all, a lot of the bacteriology I did was not immediately related to agriculture.

Hughes: Do you think he realized that when he appointed you?

Barker: Well, he appointed me because he got in touch with van Niel, with whom I had worked as a postdoctoral fellow. And then he wrote to me when I was still in Holland and offered me this position in soil microbiology. I don't know whether Dean Lipman had something to do with it, too.

Hughes: If Hoagland hired a faculty member who was going to carry the soil microbiology course, then that must have been enough to justify your position in the College of Agriculture. My point is that even though your research might not have had direct agriculture applications, you were certainly helping out the agricultural curriculum by teaching the soil microbiology class.

In those days did heads of departments or divisions have more freedom in whom they hired and the sort of research that was done? Was there less need for justification?

Barker: Oh, I don't know how they justified hiring me. [laughter] They just did. There were a number of universities in the United States where soil microbiology was given considerable support. Wisconsin, for example, was one, and Illinois, they all had courses that had been going for quite a long time and were quite successful.

Hughes: Was Lipman the first to teach it here?

Barker: Yes, I think he was probably the first one who taught soil microbiology on the campus. He enjoyed doing some sorts of soil microbiology himself. Although he was dean of the graduate school and had been I think for a number of years and continued throughout the early years when I was here, he usually would come to his laboratory in the Life Sciences Building in the morning and spend an hour or two there and then would go to the dean's office and do what he had to do there. He did that for a number of years certainly, but then when soil microbiology got started as a formal course, it was really too much work for him to

handle--to give all the lectures and take care of the laboratory and so on--and so they got me to help out.

Hughes: Well, you were at the Agriculture Experiment Station, which I presume was located in LSB. Am I right?

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Barker: I was always in the Life Sciences [Building].[9]

Hughes: Yes, but according to the records, technically your appointment was in the Agriculture Experiment Station, UC Berkeley.

Barker: Yes, that's right. Well, the Agriculture Experiment Station was a big affair. It covered a wide area of agriculture--Davis and Berkeley.

Penhoet: So it was an administrative structure?

Hughes: It was an administrative structure, yes.

Ballou: Well, where was it located on the Berkeley campus?

Barker: The dean had an office in one of these agriculture buildings, he and the assistant dean. But it was a big activity. You know, in addition to people who were teachers and professorial types, they had people who didn't have a very direct connection with the university but contact with agriculture people throughout the state.

Hughes: It still exists at the state level.

Barker: Yes, I'm sure it still exists. [laughter]

Hughes: The plant nutrition group[10]--which was your group, am I not right--was in LSB?

Barker: That's it.

Hughes: And then were there other groups in the Agriculture Experiment Station elsewhere on campus?

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[9] Dr. Barker later moved to Stanley Hall.

[10] The academic units with which Dr. Barker was affiliated underwent numerous name and organizational changes, too complicated to outline here. See his curriculum vitae at the back of this volume.

Barker: Oh, yes, in Agriculture Hall and Giannini Hall. There was food technology, for example, where [Emil M.] Mrak got his start. You know about Mrak?

Hughes: No.

Barker: Well, he was [chancellor] at [UC] Davis for a number of years.

Hughes: How did the conditions at LSB compare with your past experiences at Delft and the Hopkins Marine Station?

Barker: Well, initially the lab was a big lab with nothing much in it but a warm room and a place to sterilize glassware. There wasn't much else.

Penhoet: Who supplied the money for your research in those days?

Hughes: The Agriculture Experiment Station. I don't remember how much I got, but it was enough to get a lab started and then money was available. You see, my appointments were academic and in the experiment station. The experiment station had the money and so on and supplied what was needed.

Hughes: That would have been State of California money?

Barker: I think so. Then I also had a grant from the National Science Foundation.

Teaching

Early Courses

Hughes: Were you teaching right from the start?

Barker: Yes. I'd really never done any teaching to speak of, except for helping at van Niel's course at Pacific Grove. When I came, I didn't have the course all to myself. C. B. Lipman was in charge of the course for the first year or two, and then I sort of took charge of the laboratory work.

Hughes: And this was soil microbiology.

Barker: It was soil microbiology, in which initially there was one lecture a week which Lipman gave very informally. I usually

attended that and sort of got an idea of what he was talking about.

Hughes: What was your part in the course?

Barker: Well, initially I just went to listen to him, but I was in charge pretty much, under his general direction, of the laboratory. He was pretty busy, so sometimes he'd drop in a lab but generally that was pretty much my area.

Ballou: What kinds of experiments did you do in the teaching lab?

Barker: Well, we had a syllabus of some sort, which I worked up. Initially Lipman didn't have anything; he sort of played it by ear. [laughter]

Ballou: You would isolate organisms?

Barker: Yes. We would isolate sulfur-producing bacteria, and this, that, and the other thing.

Penhoet: How many students were there?

Barker: Well, initially there were two.

Penhoet: Two students?

Barker: Two students. [laughs] In Lipman's days classes were very small. Shortly after I came it became a required course and then we had about eighteen or twenty.

Ballou: A good way to increase enrollment is to make the course required for some major. [laughter]

Barker: Yes, well, all the students in the soils curriculum were required to take this course.

Hughes: What were their career aspirations?

Barker: Well, there were a variety of [positions] in California for people [to give] advice in the agriculture community. I think many of them went into that sort of thing. Some, of course, got out of agriculture entirely and got into other areas--microbiology. One of my best known students was Earl Stadtman, who was at the National Institutes of Health for quite a number of years.

Hughes: He did research with you as well?

Barker: Yes.

Hughes: I noticed his name on some of your papers.

Barker: Well, part of it was Ph.D. work.

Hughes: Let me go back to the teaching for just a minute. You had a very basic approach to microbiology; you were interested in the biochemistry of these organisms.

Barker: Yes.

Hughes: Did you have to change that focus when you began to teach soil microbiology?

Barker: It wasn't so terribly different from what I'd learned from van Niel. Well, there was appreciably more application to agriculture, per se. The organisms might be of some importance to the growth of plants--nitrogen fixation, nitrification, and things like this.

Hughes: Did you teach a metabolically oriented course?

Barker: Well, to some degree, but the students that we had in agriculture didn't have very extensive backgrounds in biochemistry.

The Program in Comparative Biochemistry

Barker: Later on, I developed a course with several members of the bacteriology department that was intended to teach bacterial metabolism.

Hughes: Is that the Program in Comparative Biochemistry?

Barker: Yes. I was the chief administrator in biochemistry.

Hughes: Why did you help to set up the program?

Barker: Well, we needed it for students. One or another of the [faculty] within the program had gotten many of their students [through this program].

Ballou: It's really a mechanism for going across departments to attract students.

Barker: Yes, it is.

Ballou: But it existed for more than ten years.

Hughes: Did it?

Barker: Oh, a long time. In fact, up until the reorganization of biology occurred here [in the 1980s and early 1990s].

Penhoet: But there was no biochemistry in any specific place [before 1950].

Barker: There was biochemistry but it was in the medical school [at UC San Francisco]. [David] Greenberg was chairman of the group, and there were a few other people in it.

Ballou: Right. Was it Herbert Evans whom you were thinking about in biochemistry?

Barker: No. Herbert Evans had no contacts really at all with us. He was a unit unto himself. I don't think he encouraged or had much contact, although I knew a few people who worked with him.

Hughes: Who was involved in the Program in Comparative Biochemistry?

Barker: Well, it was mainly bacteriology and agriculture.

Penhoet: Was Mike Doudoroff one of the founders of the program with you?

Barker: Yes, he for several years served as chairman of the microbiology group and the bacteriology department.

Schism in the Department of Bacteriology

Barker: The bacteriology department was strongly divided between medical and nonmedical. They didn't really talk to each other at all, to speak of! [laughter] And they taught courses that were quite unrelated.

Hughes: [Israel Lyon] Chaikoff is a name--

Barker: He was in physiology.

Hughes: He, too, was interested in getting the Program in Comparative Biochemistry off the ground?

Barker: Yes, he was one of the people who had students in comparative biochemistry.

Hughes: But he was medically oriented, was he not?

Barker: Well, he was in physiology.

Hughes: But you talked to him. [laughs]

Barker: Oh, yes. [laughs]

Penhoet: Well, I think he was talking about the schism in the bacteriology department. It was part medical people and part others, and they are the ones who didn't talk to each other.

Barker: No, there was scarcely any communication between the medical and the nonmedical bacteriologists. It was understandable; they dealt with entirely different organisms.

Hughes: Was that characteristic of bacteriology departments of that era?

Barker: I think so, yes.

Hughes: There was a schism.

Barker: Yes, in places like Wisconsin and so on they had a general bacteriology department as well as a medical school bacteriology department. [laughs] I think it was terribly common.

Research with Radioactive Tracers[11]

Collaborations with Sam Ruben and Martin Kamen

Hughes: Let's go back to your research using some of the early radioactive tracers. I understand that you worked with Kamen.

Barker: Martin Kamen.

Hughes: Actually, that was later, wasn't it?

Barker: Sam Ruben was the initial person.

Hughes: With carbon-11, right?

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[11] A discussion of tracers from the third interview session is incorporated here.

Barker: Yes.

Hughes: How did that relationship begin?

Barker: Well, [Zev] Hassid was a friend of Sam Ruben, and it was through Hassid that I got connected with Ruben. We often spent most of the night waiting for the cyclotron so we could prepare some C_{14}, and then [spent] the rest of the night [laughs] getting it in shape so we could use it. And so I guess for a time I was the only one outside--at least outside the chemistry department--that used C_{14}.

The people in the chemistry department had this nice tool that could be used and didn't really know how to use it. [laughs] They had people that I knew didn't have any background in physiology and so on so they thought it was a godsend to have microorganisms that could do all sorts of things. You didn't have to bother with patients and so on. All you had to do was take a little mud or something of the sort for a culture to do an experiment.

Penhoet: Where did the use of radioactivity as a tracer in determining biochemical patterns start? It started here?[12]

Barker: It started here. Yes, I think so. Let's see, there were some people who used heavy isotopes in other places, but as far as radioactive isotopes are concerned, it really started here.

Ballou: Martin Kamen wrote a very nice summary of this published in *Science* a number of years ago. He analyzed very precisely just where the first experiment was done and where the ideas came from.

Penhoet: So you were a natural to work on this.

Barker: [slowly] Well, yes. The two senior people were Ruben and Kamen. Kamen was the physicist; Ruben was the chemist. Initially, Kamen's job was just to prepare the isotope, to go through the procedures which are necessary.

Hughes: How did you detect the radioactive compounds?

Barker: Well, Ruben had counters, and later on I think I had a counter, too, that I used in the Life Sciences Building. But initially Ruben and Martin Kamen were the two people who were responsible

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[12] For more on the origins of the use of artificial radioisotopes, see the Bancroft Library oral history series on medical physics at Berkeley.

for the physical aspects of the preparation and counting and so on.

Ruben was an instructor, I think, at that time and maybe an assistant professor in the chemistry department. And Martin Kamen was in the Radiation Lab, but they used to collaborate. Kamen was generally involved simply in the preparation of the C_{14}, and Ruben and I were involved in doing the experiments. Kamen would come past and watch in the early hours of the morning [laughs] and see if anything would happen that was interesting.

Hughes: Nobody cared much about sleep?

Barker: Well, the only time when the cyclotron was available for people in biology was after midnight. The experiments had to be of rather short duration, too, because the half-life is only twenty minutes or so. And even if you got a reasonably strong radiation sample, it didn't last more than a few hours. You had to be all set and have rather quick methods of analysis and hope the results would be useful. The experiments often didn't have to be very refined in order to show novel results.

Hughes: This was the sixty-inch cyclotron in Crocker Radiation Lab?

Barker: Yes.

Hughes: Talk a little bit about what that was like.

Barker: Well, I didn't have a great many contacts directly with the cyclotron. It was a big box for me where they put in something, left it for a time, and it came out radioactive.

Hughes: And they handed it to you at that point?

Barker: Well, Kamen was the one who always handled the radioactive things up until the stage that they were free of other radioactive materials.

Ballou: You started with barium carbonate, correct?

Barker: Yes, we started with barium carbonate.

Penhoet: So how would you get the stuff to your lab? Would someone deliver it to you?

Barker: Oh, we did the experiments up there.

Penhoet: Oh, you did the experiments right on the site?

Barker: Yes, they were all done in that old building, which was right at the upper end of the campus. It used to be called the rat lab. I guess that's gone now.

Unison: Oh, yes.

Hughes: It's been gone for a long time.

Ballou: It was an old wooden shack of a place.

Barker: It was a three- or four-story building.

Hughes: I've heard horror stories about the lack of protective shielding and the way people handled the isotopes.

Barker: Yes, the people who prepared the isotope practically handled it with their bare hands. [laughs]

Early Tracer Experiments

Penhoet: It must have been exciting when you got the first meaningful result with radioactive carbonate.

Barker: Oh, yes.

Penhoet: Do you remember the first experiment that gave you a result?

Barker: Well, I don't remember the first one, but I remember some of the experiments.

Penhoet: What were some of the early ones when you got some positive results?

Barker: Well, we had experiments with methane bacteria in which we showed that in some situations CO_2 disappears and methane is formed. But there are other situations more commonly where there is CO_2 and you get methane, but you don't know just where it came from; it came from an organic compound, an acetate, for example.

Ballou: There was the idea then that CO_2 never got reduced back to anything; it was just the final stage in oxidation.

Barker: Oh, yes.

Ballou: If you could show that CO_2 got converted--

Barker: Yes, I think we were the first to show that CO_2 was really the source of methane.

Hughes: And that was thanks to having C_{11}?

Barker: Oh, yes.

Hughes: I think it was after World War II that carbon-14 became available for non-military uses. Didn't you do the first tracer experiment with C_{14}?

Barker: Well, I think so.

Ballou: Was this some experiment you were doing with Zev Hassid in looking at thirty steps in photosynthesis?

Barker: Well, it's all a little vague in my mind at the moment.

Hughes: It was Kamen who was largely responsible for producing the C_{14}, right?

Barker: Yes.

Hughes: And I think you did some experiments with Kamen.

Barker: Oh, yes, we did lots of experiments. Nearly all of them started late in the evening, seldom got going before ten or eleven o'clock at night. And we seldom got home before five or six or seven in the morning. You have only maybe three or four hours to do the experiments.

Hughes: [scanning Barker's bibliography] I am trying to see what you were doing with Kamen. Here we go. 1945: "Inadequacies of present knowledge of the relation between photosynthesis and the O_{18} content of atmospheric oxygen."[13]

Penhoet: [scanning bibliography] Well, here: "Carbon dioxide utilization in the synthesis of acetic acid [by *Clostridium-thermoaceticum*]."[14] That was with Kamen.

Barker: Yes, that probably was.

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[13] M. D. Kamen and H. A. Barker. *Proceedings of the National Academy of Sciences* 1945, 31: 8.

[14] H. A. Barker and M. D. Kamen. *Proceedings of the National Academy of Sciences* 1945, 31, 219.

Penhoet: Another one with Kamen: "Carbon dioxide utilization in the synthesis of acetic and butyric acids [by *Butyribacterium rettgeri*]."[15] So you really were working out the [metabolic] pathways.

Barker: Yes.

Penhoet: Hey, here's a great one: "Storage of dried fruit."[16] [laughs] Well, you did a few practical things.

Barker: Well, during the war I got quite extensively involved with people in the food technology department, doing this, that, and the other thing.

Hughes: You did some work on sugar metabolism.

Penhoet: Oh, sure, you did a lot of work with sucrose, with phosphorylase, with enzymatic synthesis of disaccharides, etcetera, with Zev.

Barker: Yes, I know.

Penhoet: Well, you spent some summers working at the C&H sugar factory, didn't you?

Barker: Oh, I was there once or twice, yes. I can't even remember what I did there. [laughter]

Barker: The Kamen research I remember best was getting up there and starting about ten at night and not finishing until two in the morning. I think that was the standard. [laughs]

Hughes: Yes, what did your family think about that?

Barker: They survived. [laughs]

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[15] H. A. Barker, M. D. Kamen, and Victoria Haas. *Proceedings of the National Academy of Sciences* 1945, 31:355.

[16] E. R. Stadtman, H. A. Barker, and E. M. Mrak, and G. Mackinney. *Industrial and Engineering Chemistry* 1946, 38:99.

Wendell Stanley

[Interview 2: January 7, 1999][17] ##

Hughes: Dr. Barker, Wendell Stanley came to Berkeley in 1948, and my understanding is that he came with the idea of uniting the various groups that were doing biochemistry around the campus. Do you remember anything about his arrival on campus and his agenda for biochemistry?

Barker: I'm afraid I don't remember very much about the early period except that it gradually became apparent that his interest in the organization was somewhat different from ours.

Hughes: How did your views differ?

Barker: Well, I had been associated with the College of Agriculture for a long time. Professor Hoagland was head of ag nutrition, and I was in that for some time, and we had become accustomed to dealing with people in agriculture and were quite comfortable, so any change was obviously going to be somewhat different and perhaps more complicated.

Hughes: What are your impressions of Stanley as a personality?

Barker: My recollections are somewhat vague about him, but I think he probably wanted to have authority over it all. Perhaps he had been told before he came that the [biochemistry] building [now Stanley Hall] would be part of his domain. So he seemed to be somewhat unhappy when people didn't consult him if any changes had to be made and so on.

Hughes: He ran into quite a bit of resistance.

Barker: Yes, he ran into resistance from time to time.

Hughes: You were in the same building with him for a while?

Barker: Yes.

Hughes: How did that work out?

Barker: Well, we didn't see each other very much, except at the Christmas parties and that sort of thing.

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[17] The second interview session has been rearranged for better topicality.

Hughes: So you pretty much carried on as you always had carried on?

Barker: Well, Stanley was on the top floor and he had a fairly formal arrangement. He had a secretary who you went to see before you could see him, and so we didn't talk to him very much. Sometimes he would initiate something, but by and large, we disregarded him most of the time. [laughter] He was probably not too pleased with that. We were in different colleges, too. He was in Letters and Sciences and we were in Agriculture. Somehow some arrangement was worked out so that we didn't have too many contacts. I'm sure that he was in somewhat of a difficult position, too. He was not interested in what we were doing; we were not interested in what he was doing. [laughs]

Hughes: The biochemical tie wasn't strong enough.

Barker: No.

Hughes: [UC President] Robert Sproul was convinced that Stanley should reunite biochemistry--you and the other groups around campus.

Barker: Which he didn't do; it fell apart.

Hughes: Yes, very dramatically it fell apart. But the disruption didn't affect your research?

Barker: Well, not very much. I suppose there were times when it seemed a little more difficult. I think on the whole we succeeded in getting along all right despite having Stanley above us, in a sense.

Hughes: He was literally on a higher level.

Barker: He was always on the top floor. We were on the third floor. [laughter]

Interaction with Campus Groups Doing Biochemistry

Hughes: Did you have any close interactions with the other groups on campus that were doing biochemistry?

Barker: Yes, I had some considerable interactions with some of the younger people in the biochemistry department, which was part of the medical school and under C. L. A. Schmidt, I think, at that time. That was in the Life Sciences Building, too. That was on the ground floor and we were on the third floor.

Hughes: Did you actually do some collaborative research? I have your bibliography. Would that help?

Barker: Probably. [laughs] I think it got started originally over the use of radioisotopes.

Hughes: Well, I know you worked with Kamen, for example.

Barker: Well, and with Sam Ruben who was in the chemistry department. Kamen, who was in the Radiation Lab, was not directly connected to any of the departments at the time.

Hughes: You mentioned that you also collaborated with the biochemists in LSB.

Barker: Well, let me see if there's anything here [in my bibliography]. Well, I had collaborations, of course, with Doudoroff, who was in the bacteriology department, and Hassid was in the same department I was in. There were several papers that were collaborations with Dr. Doudoroff.

Hughes: On a given topic?

Barker: Mostly on a given topic, namely sugar metabolism--various ways in which bacteria of different sorts handled sucrose and related sugars.

Hughes: Do you remember what you did and what he did?

Barker: Well, I must say I'm rather vague about that at the moment.

Hughes: He was a microbiologist?

Barker: Yes, he was a microbiologist. I'd known Doudoroff slightly at Stanford, not very well; I really didn't get acquainted with him until he came to Berkeley.

Hughes: Was he an undergraduate at Stanford?

Barker: He was probably a graduate student. Well, he was probably an undergraduate when I first knew him, yes.[18]

Hughes: Did he have a biochemical approach similar to yours?

Barker: Yes, he had also studied with van Niel at Pacific Grove.

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[18] Doudoroff was undergraduate and graduate student at Stanford.

The Delft Laboratory of Microbiology

Orientation towards Microbial Biochemistry and Natural History

Hughes: Van Niel's interest was in biochemical aspects of microorganisms?

Barker: Yes, right. Well, the Delft laboratory from which he came--where I spent a year as a postdoctoral fellow--had an interest both in the organisms themselves and in what they did in a chemical way.

Hughes: Was that an orientation that was characteristic of the Delft School?

Barker: I think so, yes. Much of bacteriology at that time, and perhaps still, was oriented toward medicine, and the Delft School had no orientation at all toward medicine; it was entirely toward what occurs in nature. The laboratory had developed from the work of Beijerinck. He was one of the early general soil and nature microbiologists.

Hughes: I brought you a book. Are you familiar with it?

Barker: Ah, yes. I have a copy of it somewhere.

Hughes: Dr. Barker is looking at a biography of Martinus Beijerinck.[19]

Barker: He was the great originator of the field of general microbiology in the world at that time. There were some Russians, whose names I don't remember--[Sergei] Winogradsky and some others--who were also instrumental in developing this field, but Beijerinck was very important.

Hughes: Yes, and I associate with Beijerinck's name the technology of enrichment culture.

Barker: Yes.

Hughes: Was he the originator of enrichment culture?

Barker: Well, he was certainly one of the early people who developed it more extensively than it had been previously.

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[19] G. van Iterson, Jr., L. E. den Dooren de Jong, and A. J. Kluyver, *Martinus Willem Beijerinck: His Life and His Work*, Madison, WI: Science Tech, Inc., 1983.

Hughes: Was it more difficult to get financial support for looking at microorganisms as aspects of nature than if you had been interested in them as pathogens?

Barker: Well, I don't think so. It actually might be true in general, but during that period I think it was relatively easy to get support for fellowships and so on. Van Niel had made quite a big impression by his discovery, particularly, of the photosynthetic bacteria. And the Delft School was well known, and there were various laboratories in the United States--Wisconsin and others--where general microbiology was being pursued.

Hughes: So general microbiology was a fundable area of research.

Barker: Oh, yes.

Physical Layout and Personnel

Barker: Yes, there's the building where I worked. [points to photograph of building in Delft in Beijerinck biography] It was a combination of house and laboratory. The higher part was the living quarters and so on for the professor, and the laboratory was tacked on to one lower part which doesn't really show here. But it was on the lower building which extended along the canal some little distance.

Hughes: What a wonderful setting.

Barker: Yes, it was a nice place. Boats were always going up and down the canal. It was a major commercial highway in Holland.

Hughes: Did you communicate in English?

Barker: Oh, yes. All the professors there spoke very good English. A number of the graduate students didn't, but most of them knew some English, and I gradually learned a few words of Dutch.

Hughes: What was Kluyver like as a personality?

Barker: Well, he was a very large person and outgoing man. He was a very good speaker. He gave lectures and made a good strong impression on other people, I think.

Hughes: Was he available to you in that year that you were in Delft?

Barker: Yes, he was quite available. The laboratory and his house were connected, you see. Once or twice a week he would come down to the laboratory and talk with various students and so on, but if anything important arose, you could always contact him rather readily. He had a secretary that helped him, too.

Hughes: He at that stage was mainly an administrator? He wasn't doing any bench work?

Barker: Oh, he didn't do any bench work. He probably hadn't for quite a long time.

Hughes: How old a man was he at that point?

Barker: Well, let's see, that was in the thirties. Well, I would say he was in his late fifties, maybe, or early sixties. He was very approachable, and he spoke German; he spoke Dutch; he spoke English. I remember there was an international meeting and he greeted the people who came to the lab in three or four languages. [laughs]

Hughes: Was the lab quite a crossroads for scientists interested in microorganisms?

Barker: Yes, quite a few people visited during the year I was there. I can't remember now who they all were. Oh, some people from the University of Wisconsin--[Chester H.] Werkman, I remember particularly. He was traveling in Europe and he spent two days there in Delft.

Hughes: But didn't do any research?

Barker: No. I'm a little vague now about whether there was anybody else from the United States there while I was. The Dutch speak English and German quite well. The technical people--the assistants who are paid to work there--didn't speak English very well, but I could get by all right with them with my poor German.

Hughes: How big a group was it?

Barker: The laboratory itself had about six or eight, maybe ten, rooms. Well, I can't remember in detail how many. Professor Kluyver always had one major assistant in the labs, which van Niel had been at one time. And when I was there, it was a man by the name of Kingna-Boltjes, who later became professor of microbiology in northern Holland after he left there.

Hughes: What was his area of expertise?

Barker: Well, let's see, he had done his Ph.D. on nitrifying bacteria, I believe, yes. But generally he covered the same area that Professor Kluyver did, although he was appreciably less articulate and so on. Kluyver had a very outgoing personality and spoke very well publicly as well as privately.

Hughes: Were you having scientific interchanges with Kluyver?

Barker: Oh, well, that was the main thing, yes.

Hughes: So you were talking about your research?

Barker: Yes, I was talking about my research, and other people's research which might be related.

Dr. Barker's Research

Hughes: Did you go to Delft with a specific research project in mind?

Barker: Not really, no. I decided, as I recall, to fit in with whatever [Kluyver] would suggest. And I remember, initially, that I was also interested in methanogens. I got started on the methane-producing bacteria. I'd started this in Pacific Grove, and so that's one of the things I continued on, but initially he started me out on some smaller project--some easier project.

Hughes: Do you remember what that was?

Barker: Well, I'd have to look up and see.

Hughes: Do you think you published on it?

Barker: Oh, yes, I'm sure it was published. Well, let me see. [skims his bibliography]

Hughes: You were in Delft from 1935 to 1936, so presumably the publication can be no earlier than 1936.

Barker: Yes, I think one publication was made while I was there. Let's see here. Yes, I think the first publication that I did while I was there was, "On the fermentation of some dibasic C_4-acids by *Aerobacter aerogenes*."[20]

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[20] H. A. Barker. *Proceedings of the Koninklijke Akademie van Wetenschappen te Amsterdam* 1936, 39:674.

Hughes: Why do you suppose Kluyver gave you that project?

Barker: Oh, I don't know; it was relatively easy, I think, to get started on.

But the main work that I did, so far as my future was concerned, was starting on the isolation of methane-producing bacteria. And this particular organism produced caproic and butyric acids in large amounts. And one paper was published in the *Archives of Microbiology* in '37.[21]

Hughes: Was that a significant contribution?

Barker: Yes, it was, because I think no organism producing caproic acid had been known before that time, so this was somewhat novel.

Hughes: Why would that be interesting?

Barker: Well, I suppose it's interesting because caproic acid is a six-carbon compound and butyric acid has four carbons. And I suppose it extended the range of chemistry. Also caproic acid apparently had some uses which butyric acid didn't have. Butyric acid and butyl alcohol had been produced commercially previous to that time, and the organism that I isolated was used in Delft later on, I think, for caproic acid production--maybe something else, but I'm not sure what now.

Hughes: What is caproic acid used for?

Barker: Well, as a compound it just has two more carbon atoms [than butyric acid].

Hughes: Yes, but does it have some industrial use?

Barker: Well, evidently it did have some, or Professor Kluyver thought it might.

Hughes: You, I'm gathering, were not particularly interested in the practical applications of this work.

Barker: No.

Hughes: You were interested in how these organisms functioned in nature?

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[21] H. A. Barker. "The production of caproic and butyric acids by the methane fermentation of ethyl alcohol." *Archiv für Mikrobiology* 1937, 8:415.

Barker: Yes, but you know I was a young person getting started. To have something that is of some interest outside of the laboratory is also nice. [laughs]

More on van Niel

Hughes: Do you think, in terms of the university, that your connection with van Niel and Hopkins Marine Station probably meant more than your connection with Kluyver and the Delft School?

Barker: Oh, very likely. Van Niel was very well known. He had a very outgoing dramatic personality, really, and he had done very good work. And I guess the fact that I had studied with him for several years and then had gone to Delft and studied there was--they thought I ought to have a good background. [laughs]

Hughes: Yes, I would think! From the best. Compared to other students at your level, didn't you have much more experience? You had spent time with van Niel.

Barker: Well, I'm sure I had more experience than some because I'd already had some Delft experience. [laughs] Van Niel's laboratory in the days when I worked with him was set up very much on the pattern of the Delft laboratory.

Research on Photosynthetic Bacteria

Hughes: Why would a microbiologist be at a marine station?

Barker: Well, that's a good question because it really is sort of chance, I think. There are, after all, lots of microorganisms in the ocean and in the vicinity of the ocean, and so obviously this is a place where one might come in contact with organisms that you wouldn't find in another environment.

Hughes: Is that particularly true of the photosynthetic bacteria?

Barker: Probably not. All the photosynthetic bacteria actually came from the land--ponds and so on. Photosynthetic bacteria are anaerobes, most of them, and they don't like a lot of oxygen, and so they wouldn't thrive in the ocean.

Hughes: When you fly over the salt marshes coming into San Francisco, isn't the red color due to *Halobacterium halobium*?

Barker: Well, there probably is a genus of that name down there.

Hughes: I was wondering if that was one reason that van Niel was at the marine station.

Barker: The ones that I'm familiar with are *Rhodospirillum* and the *Halospirillum*. I'm not sure if *Halospirillum* is photosynthetic--probably not. It probably just means that they are salt tolerant.

Lectures

Hughes: Did you find similarities in the science that was done at Delft and Hopkins Marine Station?

Barker: Oh, yes. Van Niel was a very good student of Kluyver's, and actually [van Niel] was probable a more impressive teacher, as a lecturer and so on. His lecturing was very intense and very dramatic, whereas Kluyver gave a good informative lecture, but there was less passion involved in the presentation. Oh, van Niel was an extraordinary person from the point of view of lecturing and presence. I think everybody who came under his influence was really impressed.

Hughes: Was he largely lecturing from his own experience?

Barker: Oh, no, he was mainly lecturing on the basis of history. He worked very hard in bringing historical information into his courses and so on.

Hughes: There was already substantial information on these microorganisms?

Barker: Well, on some of them there was and for some there wasn't. [From] people like Winogradsky and Beijerinck and so on.

Hughes: Did van Niel ever teach on the Stanford campus?

Barker: Yes, he did. He used to come up in the spring for several years --I don't know how many--and did his series of lectures without even a laboratory. That is, it was just demonstration of organisms and so on. He didn't like that as much, but apparently it was considered desirable at the university so he did it.

Hughes: He had no ambition to be on the Palo Alto campus?

Barker: No, definitely not. The marine station was home. He had a very nice house in Carmel and a short drive over to the marine station. The marine station was a nice place. When he first went there, well, there had been some disruption; people of several sorts were there-- invertebrate zoologists and so on. In fact, the director of the marine station in the early years when van Niel was there was an older man interested in invertebrate zoology. I can't remember his name.

[Barker's comment lost in tape change] ##

Hughes: What was the electricity being used for?

Barker: Well, for heating water baths and that sort of thing. The budget of the marine station was pretty tight in those days. They used to suggest that we use the minimum amount of electricity.

Hughes: Did the invertebrate zoologists stay around for van Niel's career?

Barker: No. During the Depression, the marine station essentially shut down except for van Niel's laboratory. Several other people who had been on the staff left or discontinued for shortage of funds and so on. But van Niel thrived during that period and gradually built it up again.

Hughes: Did the invertebrate zoologists return once the Depression was over?

Barker: Well, I'm rather vague about what happened down there.

Hughes: I was wondering if it became a van Niel operation.

Barker: Yes, that was the major activity. There was some oceanographic work. They had a boat that was used. One member of the staff used to go out fairly regularly early in the morning and collect some data on temperatures and this, that, and the other thing. But gradually it stopped.

Hughes: So it really became--

Barker: A big microbiology place, for the most part. Although, in the summertime, some of the people from Stanford came down there and did a little research with invertebrates and so on. And there were a few students in the summer, and in the wintertime there were probably half-a-dozen people.

More on Dr. Barker's Arrival at Berkeley

Soil Microbiology

Hughes: Do you think that your work on the methane-producing bacteria was one of the reasons that the University of California at Berkeley became interested in you?

Barker: Oh, I doubt that very much.

Hughes: Why?

Barker: Well, I don't know. Nevertheless, it's always possible that they thought this was a new area and that if I got into this area I might get into others that would (_____). [laughs]

Hughes: Why do you think they were interested in you?

Barker: Well, before I came here, the teaching of agriculture bacteriology essentially was done by C. B. Lipman, who was dean of the graduate school. He had taught this [course] sort of in his spare time, which wasn't very great because he was pretty busy with his deanly duties. I guess they thought it would be desirable to get somebody to relieve him of his teaching responsibility in this area.

Hughes: And really do a concerted job of teaching soil microbiology?

Barker: Yes. The class was very small before I came; I think he just had one or two students at a time. But later on it became a required course for students in the soils curriculum, and I think there was normally something like fifteen or twenty students. It wasn't a large subject--large subject but not a large student [demand].

Hughes: You were located in the Life Sciences Building which, I understand, had problems, the physical plant itself. I've heard stories about the rat infestations, for example.

Barker: Well, occasionally one saw a rat. Not very often.

Hughes: So that wasn't really a problem.

Barker: Not for me.

Faculty

Hughes: Do you remember who was there when you arrived as a young faculty member?

Barker: Well, Hoagland, of course, and Lipman.

Hughes: Was Hassid there?

Barker: Yes, he was a graduate student when I first came. I was closely associated with Hassid. Later, I think, he was part of the department, too. And Doudoroff came fairly early [1940]. I think he came a few years after I did. And then there was Roger Stanier. They were both in the bacteriology department which was close by in the Life Sciences Building, just up a floor.

Hughes: Hassid was actually in plant biochemistry?

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Barker: Yes, in plant nutrition. Some of the people had academic appointments. I think nearly all of them had also an experiment station appointment. But then there were also people who only had experiment station appointments, who basically were assistants to Professor Hoagland. He was chairman of the department.

Hughes: You had appointments of both kinds--on the faculty and in the experiment station.

Barker: Yes. And when I first came there, Dean Lipman also came there from time to time and carried on some experimental work.

Hughes: But not directly with you?

Barker: Not directly, no. We were quite closely associated. We used the same facilities for sterilization and cold and warm rooms and that sort of thing.

Equipment for Microbial Research

Hughes: Was LSB well-equipped for microbial research?

Barker: Well, fairly well. I added to that after I came. I think we got a big walk-in cabinet for greater variety of temperatures because

[before] there was just a single warm room there which was kept at 30 degrees. If you wanted some other temperature, you had to devise them the [best you could.] [laughs]

Hughes: So you provided a facility that had a range of temperatures for use with cultures?

Barker: Yes, the temperature could be adjusted to the way we wanted. It was a walk-in thing, but it was not very big. [demonstrates size] And inside there were various shelves.

Hughes: So about eight feet by eight feet, would you say?

Barker: Well, it might have been a little smaller than that--maybe six feet by eight feet.

Hughes: Did you introduce enrichment culture to this group or was that something that most microbiologists knew about?

Barker: Well, enrichment culture is something much older, but I think I extended this to a greater variety of organisms and so on. Well, of course Lipman was familiar with all the classical work that had been done in microbiology, which started with [Louis] Pasteur, I suppose, and then there was Winogradsky.

More on Enzymatic Synthesis of Sucrose

Hughes: Research that certainly got some attention was on vitamin B_{12} coenzyme.

Barker: Ah, yes.

Hughes: Do you remember how you got into that?

Barker: Well, I'm a little vague about it at the moment.

Hughes: Do you want to look at your bibliography?

Barker: [skimming his bibliography] Well, we got into the enzymatic synthesis of sucrose, I remember.

Hughes: That was wartime work?

Barker: Oh, no. That made quite a big impression, because the sugar industry had some vague interest in this.

Hughes: Did you have support from the sugar industry?

Barker: Yes, I think so.

Hughes: Were you working on a specific organism?

Barker: Well, let's see, I must say I'm very vague about much of this.

Hughes: Just run your eye down the page and see if anything comes to mind.

Barker: The first paper is, "Enzymatic synthesis of crystalline sucrose," for which I was senior author and Hassid and Doudoroff were also authors.[22]

Hughes: Do you think you got into the sucrose work because your interests led you in that direction? Or do you think that the sugar industry might have prompted your interest?

Barker: Oh, no, I had no interest in the sugar industry, that was pure coincidence. No, they were interested in it.

Hughes: How did they get to know about you?

Barker: Probably from the papers that were published. Well, it's possible that we contacted somebody for some reason or other.

Hughes: More likely that they contacted you.

Barker: I'm rather vague about that now.

Hughes: Dr. Penhoet thought that you had spent some time in the summer working for C&H Sugar.

Barker: I remember at some stage I visited one of their plants, but I must say I'm quite vague about this now. But basically it was something that we got started in the lab first.

Hughes: Yes, and that was true all the way along, wasn't it? You followed your research where your interests led you.

Barker: Well, I never got deeply involved in any commercial interest. I'm afraid I'm not being a very good [respondent].

Hughes: Well, it's a long time ago. Turn the page of your bibliography to the B_{12} research.

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[22] H. A. Barker, W. Z. Hassid, and M. Doudoroff. *Science* 1944, 100:51.

Barker: Oh, that was later, I believe.

Hughes: Yes, that was mid to late-fifties, I think.

More on Research with Radioactive Tracers

Access to the Cyclotron

Barker: I see there was some C_{14} work on CO_2 utilization.

Hughes: That was with Martin Kamen.

Barker: Yes. That had quite an influence, I remember, on what I did for some time.

Hughes: In what way?

Barker: Well, this [using radioactive tracers] was a way of finding out things that you couldn't find out very readily any other way, so we did a number of experiments. It was always a little awkward because usually the cyclotron was only available [to biologists] from about midnight to two a.m., so we got started usually about maybe ten or eleven at night and usually then had to wait until Kamen could get a hold of the cyclotron which was used for lots of other things, of course, during this period. So we got it when nobody else was using it.

Hughes: The biologists were the low men on the totem pole. [laughter]

Barker: Very low.

Tracer Use of Carbon-14

Hughes: Were any other microbiologists using radioactive tracers in their work at that time?

Barker: Well, some people were beginning to use C_{14}, which was the long-lived radioactive isotope. See, where we had a twenty-minute half-life isotope [C_{11}] which made it possible to do quick experiments, the people in the East had the long-lived radiocarbon which they could work on indefinitely.

Hughes: They had C_{14} before Berkeley?

Barker: Yes, [pauses] I think so. There was a cyclotron at the University of Chicago.

Hughes: So perhaps they were the first to use C_{14} in metabolic studies of bacteria?

Barker: I don't know that they were the first ones. We may have been the first ones because we had the organisms that were already pretty well known that probably they did use CO_2 in their metabolism.[23] But others also had problems that could be solved with the use of isotopes fairly early.

Hughes: Also, you had Martin Kamen right here on campus. Wasn't he one of the very few who could prepare C_{14} for tracer use?

Barker: Oh, yes, he was initially. All experiments were done with Martin. [laughs]

Hughes: Well, who knows, you may have been the first. [laughs]

Barker: Well, I think perhaps we were the first to do experiments of that sort, but then other people came along rather soon thereafter.

Ernest and John Lawrence

Hughes: Ernest Lawrence, as I understand, was quite interested in having radioisotopes used in biological research.

Barker: Well, I think he thought that it would be good for the cyclotron and his general activities to have people working on other approaches to biology. But we had virtually no contacts with him at the university; I think I only met him once or twice on committees. He was never up at midnight or two a.m.

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[23] In his "Notes on the history of biochemistry at Berkeley" (1969), Barker writes of a paper he co-authored in 1941 which reported "the first use of C_{14} discovered the previous year by Ruben and Kamen (1940) as a tracer in biological system[s]." See appendix to oral history.

Hughes: Oh, I see. [laughter] What about his brother, John Lawrence?[24] Was he around at all?

Barker: No, he was not. A little later on he did some experiments, I think, with carbon isotopes, but it was entirely unrelated to the sort of work that [we were doing].

Hughes: Right, he was a physician and he was more interested in clinical application.

Barker: Yes.

Hughes: I just thought maybe your paths had crossed.

Barker: Well, I met him on one or two occasions.

Tracer Research with Martin Kamen

Hughes: So when you were doing these experiments, it was Martin Kamen and you--the two of you?

Barker: Yes. I'd meet him about midnight or so and usually go on from there.

Hughes: You would bring your cultures to the Rat Lab?

Barker: Yes, I'd have to get ready and get cultures to the proper stage so we could do the experiments. And then it was just a question of taking the cell suspension and the appropriate nutrients and then putting in some radioactive carbon and waiting a short time and then analyzing the results--see where [the carbon] went and how much got into what.

Hughes: And the radioactive carbon was in the form of CO_2?

Barker: Initially it was provided entirely in the form of CO_2. It's possible we did some experiments with some other compound than CO_2, but I'm a little vague about this now. Probably if I looked through [the bibliography] I could find out.

Hughes: You think that maybe you also used a solid substrate which incorporated radioactive carbon?

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[24] See the oral history with John Lawrence in The Bancroft Library oral history series on medical physics at Berkeley.

Barker: No, but we might have converted the CO_2 into some other compound and then used that as the radioactive source. [continuing to scan bibliography] One of the experiments was on CO_2 utilization for making acetic acid,[25] and then making butyric acid and caproic acids, plus we did *kluyveri*.[26] Well, it was a long time ago.

Hughes: It was. Your particular interest was anaerobic soil microorganisms?

Barker: Well, yes. Actually, much of my work was with anaerobic organisms, and so they were the ones that were readily available for doing these various experiments.

Hughes: Were they easier to work with than aerobes, is that what you're saying? Presumably aerobic bacteria were also available, were they not?

Barker: Yes, but the ones that I was working with at that time were mostly anaerobes, so we happened to have material--

Hughes: That was what you had. [laughter]

Barker: That's what we had and that's where the opportunities of C_{14} became more apparent.

Research on B_{12} Coenzyme

Hughes: The B_{12} coenzyme work attracted the interest of industry.

Barker: Yes, I guess that was the aspect of my work that was most interesting to industry.

Hughes: Did the interest of industry make any difference in the way you did your research?

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[25] H. A. Barker, S. Ruben, and M. D. Kamen, "The reduction of radioactive carbon dioxide by methane-producing bacteria. IV. The synthesis of acetic acid from carbon dioxide by *Clostridium acidi-urici*," *Proceedings of the National Academy of Sciences* 1940, 26:477.

[26] H. A. Barker and S. M. Tata, "*Clostridium kluyveri*, an organism concerned in the formation of caproic acid from ethyl alcohol," *Journal of Bacteriology* 1942, 43:347.

Barker: Oh, I don't think so, not appreciably, no.

Hughes: You just carried on and they fit in?

Barker: Yes. [laughter]

Hughes: Although you do have a patent on the process for making B_{12} coenzyme.

Barker: Yes, I have a patent. That happened later over the years [1962].

Hughes: Do you remember whose idea it was to patent the B_{12} coenzyme work?

Barker: No, I can't tell you that at the moment, but probably the industrial people were interested in that.[27]

Hughes: You wouldn't have initiated patenting?

Barker: Well, I don't think so. I may be wrong. I might have at that time. [laughs] Maybe I thought I would get a little something out of it in terms of monetary reward. And I probably did get a little, but not very much.

Microbial Culture Collections

Barker's Collection at Berkeley

Hughes: Did you have large culture collections as time went on?

Barker: Oh, not very large, no. Nearly all of experiments of this type were done with a few [species of] organisms.

Hughes: Because that's all you required?

Barker: Well, they did what we were interested in doing.

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[27] In March 1960, Dr. Barker wrote to a scientist at the Squibb Institute for Medical Research in response to his letter about patent coverage of various coenzyme analogs. (H. A. Barker to David Perlman, March 3, 1960. Barker correspondence, Bancroft Library, CU467, box 6, folder 49.) On May 29, 1962, the U.S. Patent Office issued a patent to Barker, assigned to the UC Regents, for "B_{12} Coenzymes and Processes for Preparing the Same."

Hughes: Yes, so you didn't need more types.

Barker: No.

Hughes: But when you finished a project--say, the sucrose project--would you continue to culture those organisms, or was that the end of it and you'd toss them out?

Barker: Oh, no, these were mostly organisms which we had worked with and probably are in collections somewhere around the country in addition to our own lab. What one generally does, if you have an organism with some interest is you send it to the American Type Culture Collection, and then they have the trouble of keeping it from then on. I don't know whether they still have those organisms or not. Very likely, some of them they still have.

Hughes: So once you had finished with a particular organism, there was no particular reason to continue its culture in your laboratory?

Barker: Well, unless we had other [interests].

Hughes: Yes, right. Because if you needed it again, you could write to the American Type Culture Society.

Barker: Yes, but usually you would keep around the organisms that you're familiar with if it isn't too much trouble. And often some of these organisms were spore-formers, so you could grow them up until they formed spores and then dry the material and they would last more or less indefinitely. When you wanted one, all you had to do was go back to this powdered material. But others that don't form spores you had to transfer fairly regularly. It becomes a problem to do it, so for a lot of cultures it's better to let the American Type Culture Collection do this sort of thing.

Hughes: Had that been started way back?

Barker: Yes, I don't know just when it was started.

Hughes: But it was available throughout your career.

Barker: Yes.

The Culture Collection at the Microbiology Laboratory, Delft

Barker: Delft had a big collection, too. Actually, van Niel's job as a graduate student was taking care of the culture collection of Delft. Professor Kluyver, at least in the time when I was there, never did any hands-on work. He was the idea man. And then van Niel would help to write up papers and all that sort of thing. See, van Niel had been his right-hand man in the laboratory.

I think that was the system that Kluyver always used. He had some senior student who for several years was under him and was sort of responsible for seeing that everything went right and giving advice to people and so on, so that eased the amount of work Kluyver had to do and direct contact with students. After all, Delft Laboratory was just a one-man [operation].

Hughes: It was Kluyver, wasn't it?

Barker: It was Kluyver. And he had a pretty big laboratory at times. He had maybe ten to twenty students there, and he had lectures to give, and outside activities, I'm sure, of different sorts, so he needed help.

Hughes: He lectured at the university?

Barker: At the university, yes.

Hughes: Right, so he was a university professor that happened to have--

Barker: He was a single department in himself, that's what it amounted to. There wasn't anybody else.

Hughes: Yes, and the department was the building that you showed me, which wasn't, of course, on a campus.

Barker: Well, I think that was generally true at that time; individual professors just had their own establishments, rather than having several rooms in a big university building. So pieces of the university were spread around.

Hughes: Were the cultures that the Delft laboratory maintained readily available to scientists elsewhere?

Barker: Yes, I think they were. I think one could write Professor Kluyver, and he'd get somebody to send them cultures, yes.

Hughes: Did you also do the same thing once you were established at Berkeley?

Barker: Oh, yes. Yes, we had a modest culture collection.

Hughes: Even though the American Type Culture Society was available, people would still write to you?

Barker: Yes, the organisms you're working on, you want readily available whenever you want them.

Hughes: But people who were not at Berkeley would nonetheless write to you for organisms?

Barker: Yes, sometimes.

Hughes: Why wouldn't they write to the American Type Culture Society?

Barker: Well, they often did that, too, I'm sure. [laughs] Or to van Niel's laboratory. He had quite a good-sized culture collection. I never had as large a collection as he had, but he followed the sort of Delft pattern. I don't know, he probably had maybe hundreds of cultures which he kept which he used partly in connection with his teaching and partly in connection with research.

Hughes: Were there any stipulations attached to giving out these organisms? Any limitations in how they might be used or credit that had to be given?

Barker: Well, I think one generally acknowledged, if you got a culture from somewhere, where it came from, sort of to establish what it was, its authenticity.

Hughes: But it was quite different from the system now, where a scientist writing for use of a specific plasmid, for example, has to assure the donor that he's not going to use it for commercial purposes. There was none of that?

Barker: No, I suppose commercial use was less prevalent then than it is now.

Van Niel's Collection

Barker: Van Niel basically had a substantial part of the Delft collection--that part that he was interested in at any rate. Delft, for many years, from the early years of Beijerinck, had developed this collection; somebody in the laboratory had to take

care of it. While van Niel was a graduate student, he for several years was responsible for transferring the collection and so on. That responsibility went to somebody else after he left.

Hughes: He came to this country with some of that collection?

Barker: Yes.

Hughes: And I'm sure added greatly to it.

Barker: Yes. I don't know, he must have had some hundred or more--hundreds maybe--cultures that he kept. He had a special assistant whose job was to do the transferring and so on, among other things. He prepared media for his classes and that sort of thing.

Hughes: So he had a pretty nice setup at Hopkins Marine Station?

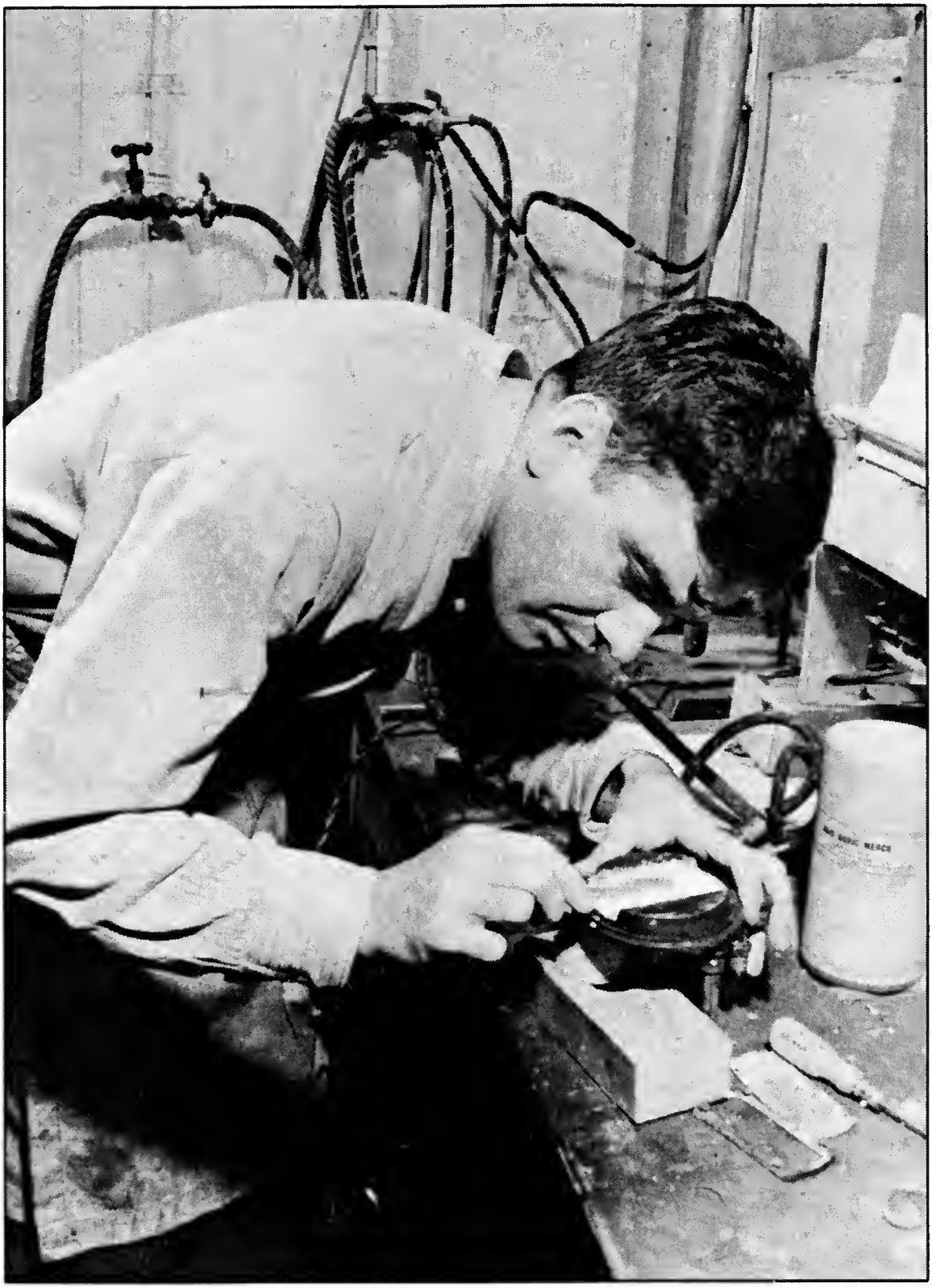
Barker: Well, it was very nice and very good for him; it was quite small. He was the microbiologist, and he had one full-time assistant. I don't know what her training had been. She might have had some university experience, probably didn't graduate from a university, although she might have. So he taught her what she needed to know.

Hughes: She was his right-hand woman, so to speak?

Barker: Well, she took care of the culture collection, yes, and certain things around the laboratory.

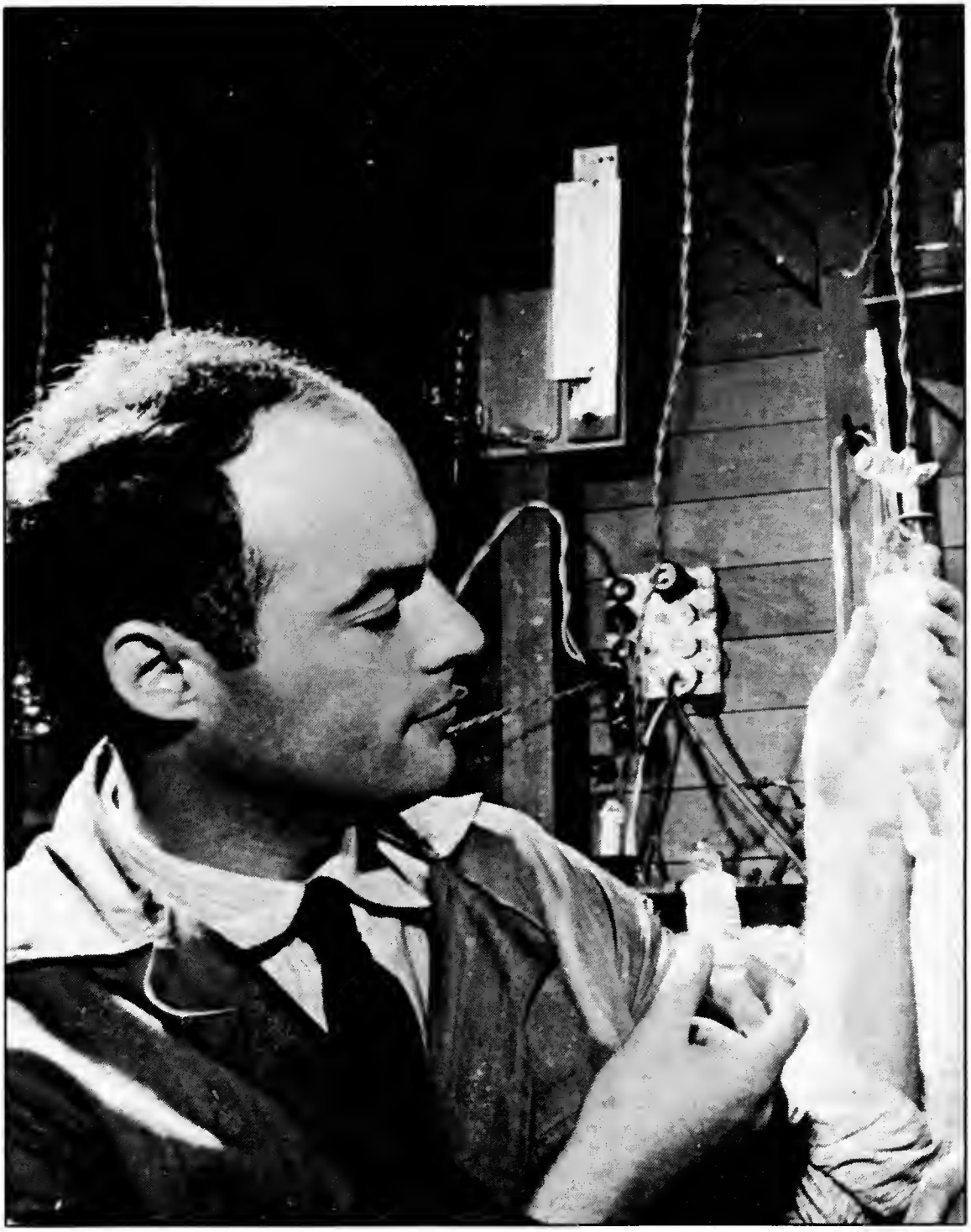
Hughes: She was with him for a long time?

Barker: Yes. I can't even remember her name, but she was there for quite a number of years.

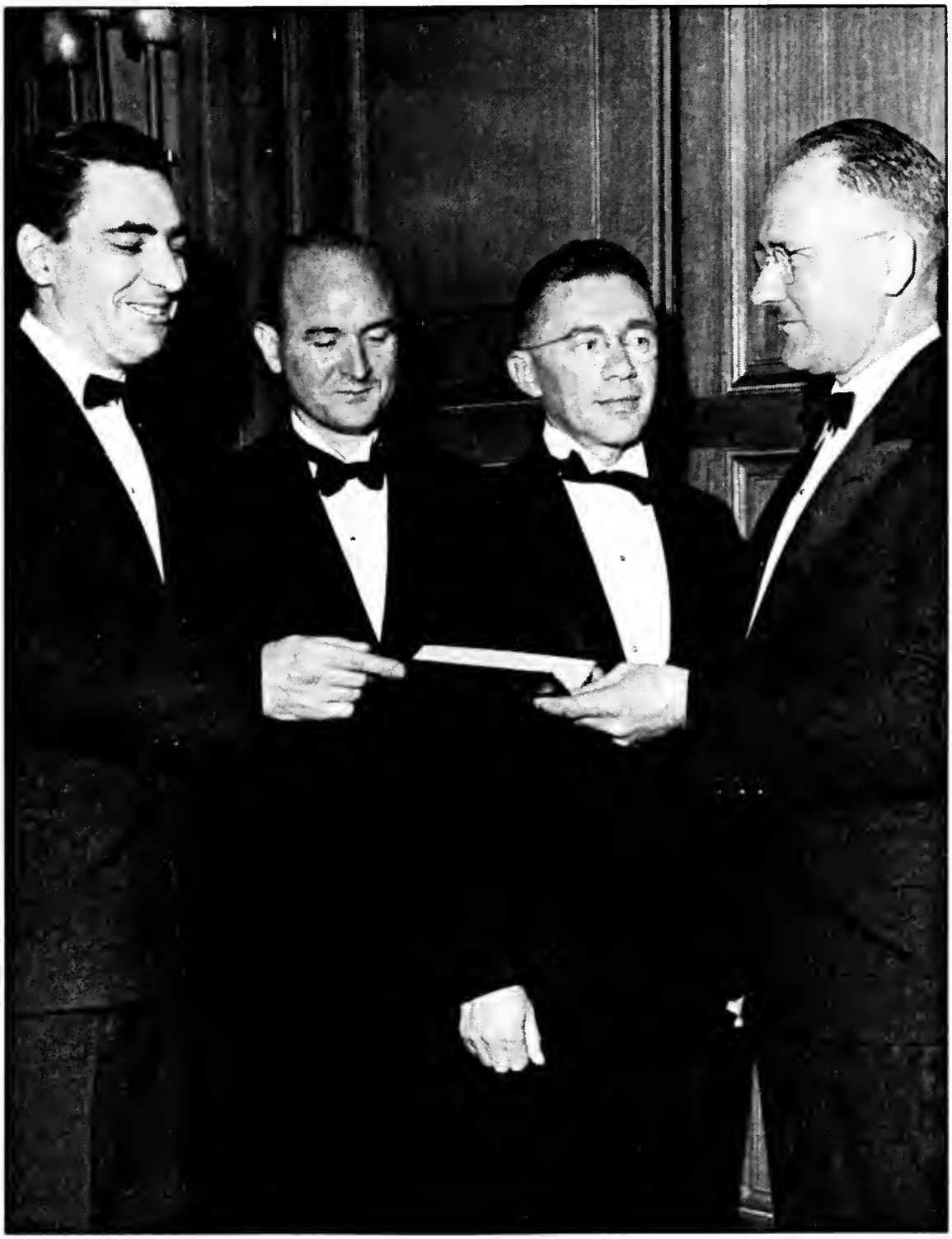


Martin Kamen preparing carbon-11 target, circa 1940.

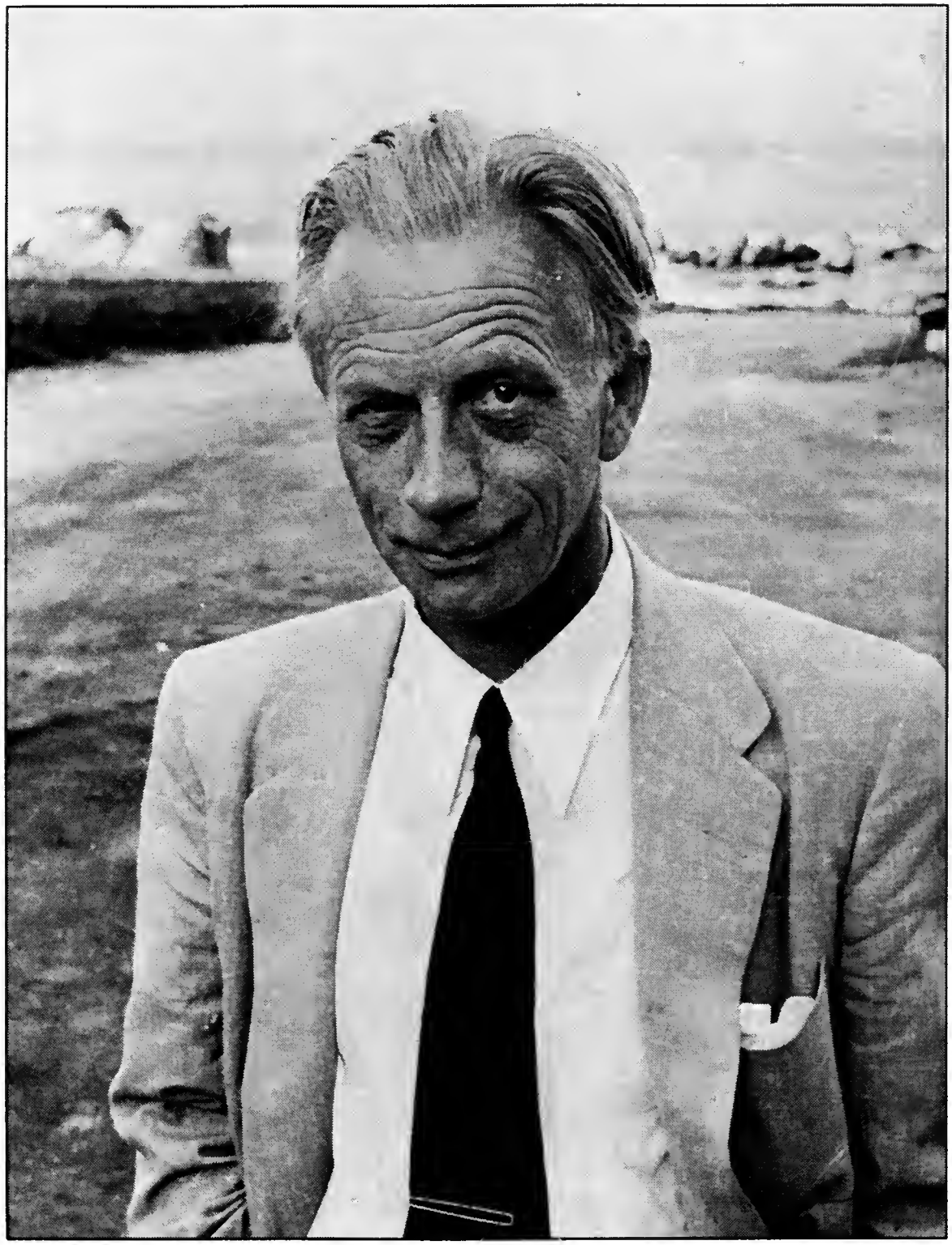
Martin Kamen operating the cyclotron at the Radiation Laboratory, circa 1940.



Sam Ruben, circa 1940.



Michael Doudoroff, Horace Barker, and W.Z. Hassid receiving the first Sugar Research Award from George Beadle, 1945.



Cornelius B. Van Niel, circa 1948.

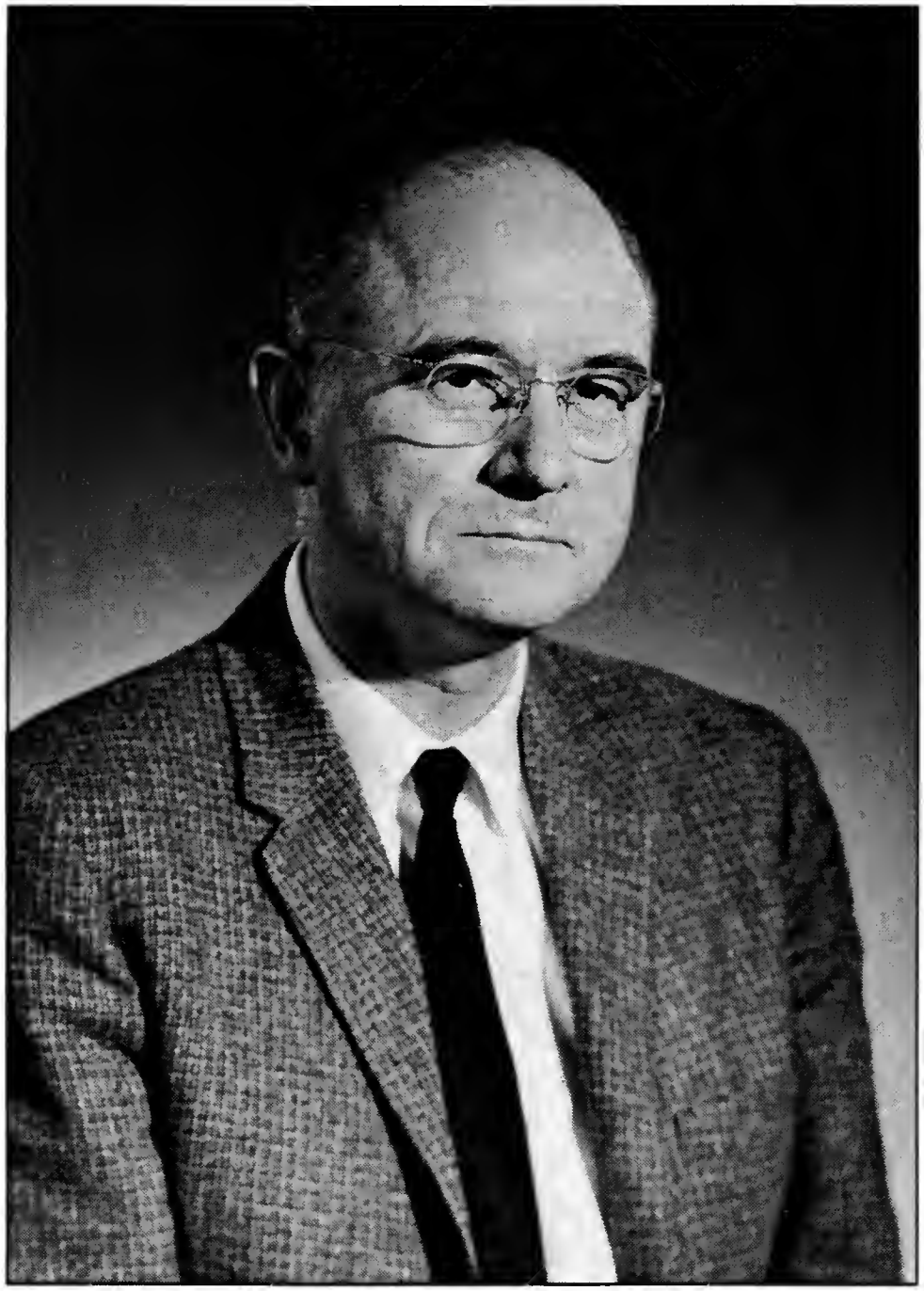
Photo courtesy V.B.D. Skerman, University of Queensland.

A. J. Kluyver

Photo by Studio Odijk, Delft.

Department of Agricultural Biochemistry, 1957.

Photo by Barry Evans.



Horace A. Barker

More on the Program in Comparative Biochemistry

[Interview 3: January 14, 1999] ##

Faculty Participants

Hughes: In 1946, you and I. L. Chaikoff organized an interdepartmental graduate group major, the Program in Comparative Biochemistry.[28]

Barker: [It was a way] for anyone in the [interdisciplinary graduate] groups to work with somebody in one of the others. It was for the times fairly useful.

Hughes: Was the medical school [UCSF] biochemistry group included?

Barker: Yes. For example, Chaikoff and Greenberg at this time were in the medical school. They had just recently been transferred from whatever they were in before into the medical school curriculum. Hassid and I were in [the Department of] Plant Nutrition. I was in what was called Plant Biochemistry, but it was under Plant Nutrition. And then [Maynard Alexander]Joslyn and [Gordon] Mackinney were in Food Technology. We collaborated to some degree with them for a number of years, particularly during the war years. Nearly everybody left and those who remained worked on war projects of one sort or another.

Hughes: Was that by choice?

Barker: Well, by choice, yes.

Hughes: You wanted to help the war effort?

Barker: Yes, it seemed like there were some things that we could do that might be slightly useful. [laughs]

Hughes: But then when the war was over you went back to your comparative biochemistry?

Barker: Well, I think the Comparative Biochemistry [Program] continued during this period.

Hughes: Do you remember whose idea it was to form it?

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[28] Memo, May 11, 1946 (Barker papers, Bancroft Library, CU-467, folder 60).

Barker: It was my idea.

I. L. Chaikoff and the Medical School

Barker: But Chaikoff was quite interested because he was in the medical school, and he liked to have students that weren't in the medical school, people interested primarily in the biochemical problems that he worked on.

Hughes: He was a basic scientist?

Barker: Yes, he was a basic scientist. He was interested in a combination of physiology and biochemistry.

Hughes: Did he regret having to be transferred to the medical school?

Barker: Oh, I don't know now if I can say he regretted it or not, but obviously it made some complications in handling [graduate] students. He had some that I'm sure were not really interested in medicine per se, and so it made it easier to handle those in interdepartmental [graduate] groups, such as this one.

Hughes: I imagine that there were also students who were really only interested in medical problems?

Barker: Well, there may have been some.

Hughes: I mean, once he got over to the medical school in San Francisco.

Barker: Well, he didn't go to the medical school. Part of the medical school remained in Berkeley in the Life Sciences Building for a number of years. Ultimately it was all transferred, but I think parts of bacteriology, parts of physiology, and parts of biochemistry were connected to the medical school while they were still in Berkeley. I don't think Chaikoff ever was in San Francisco. I think he retired in Berkeley.

More on Faculty Participants

Barker: Greenberg, I think, did go to San Francisco for a time. [referring to 1946 memo re faculty participants in the graduate group] [Paul] Kirk: his primary interest was in microchemistry. He had interests outside the university in consulting of various

sorts. I don't remember now which group he stayed with. [Sherburne F.] Cook I think was in Physiology. Hassid and I were in Plant Nutrition under Professor Hoagland, and Joslyn was in Food Technology. He was in one of the buildings along the north side of the campus, before the big buildings right along the street were built. Food Technology, Plant Pathology, and several other small departments were located in that building, if it's still there. [Harold] Tarver was in Biochemistry and Mackinney was in Food Technology, Doudoroff was in Bacteriology, and [Samuel] Lepkovsky was sort of by himself up the canyon.[29]

Hughes: At LBL [Lawrence Berkeley Laboratory]?

Barker: No, there was an old laboratory--I'm not sure if it still exists --which was above the Biochemistry Building up the canyon a ways. There was a road that went up there past the tennis courts and the swimming pool and ultimately the road turned up and went up to the Radiation Lab up on the hill, and there was a whole building there that was very antique.

Hughes: And no longer exists?

Barker: Well, he got along there until he retired and after he retired I think it was probably torn down.[30]

Student Participation

Hughes: I read somewhere in those documents that there were eleven different departments represented in the Program in Comparative Biochemistry.

Barker: Oh, yes. It presumably took in most of the biology departments, and some of them I think probably never had any students in this area and some of them had several. I think Food Technology had several students, Bacteriology had some, Plant Nutrition had some. Well, there may have been some others, too. It was not a large group.

Hughes: Was there ever a problem in the different agendas that I presume both students and professors brought to the group? A field such

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[29] Dr. Ballou notes: "Lepkovsky was in Nutrition, worked on poultry husbandry and had a lab on what is now called Cenetennial Drive. The building is still there and is devoted to Atmospheric Aerosol Research."

[30] Louise Taylor believes the building may have been used thereafter by Poultry Husbandry and then torn down recently.

as Food Technology, for example, has a practical orientation, but what you were doing was very basic research. It worked to have an umbrella group?

Barker: Well, when it came to a Ph.D. examination, one put people on the committee who were appropriately there. [laughs]

Hughes: So it was a large enough group that you picked and chose according to the needs of the dissertation committee?

Barker: Well, the students really chose us rather than we choosing the students. It was designed for students who didn't quite fit in the more specialized requirements of a particular department. They were required to take a variety of general courses--bacteriology and biochemistry--and then they always took some more specialized courses--in physiology, and so on.

Hughes: Did all of your students work through this graduate group?

Barker: No, I had some students that got degrees in microbiology, which was also an interdepartmental group. Well, [our comparative biochemistry] group was really designed to take care of students who for one reason or another didn't fit in with a particular department, whose interests in terms of the standard departments were sort of interdepartmental. It was not very large. I think we never had more than six or eight students at one time, but it lasted for a number of years.

Hughes: You will see in those documents a listing of the course requirements.

Barker: Here we go. So we had Chaikoff, Cook--what department was he in?

Hughes: Is that Sherburne Cook?

Barker: It's S. F. Cook.

Hughes: I'm almost sure he was in the Department of Physiology.[31]

Barker: I think he was, too. He wasn't a very prominent member [of the Program in Comparative Biochemistry]; I don't think he was there very long, either.

Hughes: I know his name because he, like you, was one of the early users of radioisotopes. But not as early as you were.

---

[31] Cook joined the department in 1928 as an assistant professor.

Seminars

Hughes: I also noticed some reference to seminars that were supported by the graduate group as a whole. Do you remember that? These seminars were different from those that presumably each department sponsored.

Barker: Well, we did for a number of years have seminars in general microbiology. I think Mike Doudoroff and Roger Stanier were in charge of that at one time. I think we would each take turns finding people who would be willing to talk.

Hughes: Those seminars were for the Department of Bacteriology or were they for the Program in Comparative Biochemistry?

Barker: Well, I think it was the Program in Comparative Biochemistry. The senior people in bacteriology were rather medically oriented, except for the people who had come from van Niel's laboratory, and so they, instead of combining with the medical people, had their own seminar series which was on nonmedical subjects.

Hughes: So that was another purpose of the program; it allowed you to explore subjects that might have been a bit more difficult to explore, at least on a seminar basis, in specific departments.

Barker: Oh yes, I think so.

Hughes: Was there anything else that the Program in Comparative Biochemistry allowed one to do?

Barker: Well, it mainly functioned to handle graduate students who did not want to get directly involved in the medical biochemistry departments, and there were quite a lot later on. I don't know if any of them are here. Let's see, there are one, two, three, four--[continues counting to fifteen before interrupted]

Hughes: Are you counting up students who--

Barker: Who I had.

Hughes: In the Program in Comparative Biochemistry?

Barker: Yes.

Hughes: You wrote in an article in the *Annual Review of Biochemistry* that seventy-five students majored in comparative biochemistry.[32]

Barker: No, those were not all mine because this included people in the bacteriology department under Stanier, Doudoroff, and [Edward] Adelberg, and I think there were maybe even one or two in other departments.

Hughes: That's quite a number of students.

Barker: Well, it was the time when general microbiology, and biochemistry related to it, flourished in Berkeley.

Hughes: Those years were the high points?

Barker: Yes. Doudoroff was very much interested, for example, in problems related to sucrose and so on, and I was involved in this somewhat also. We got a lot of publicity on bacterial synthesis of sucrose, not that it ever had any practical application; it was theoretical.

Hughes: Yes, I saw some newspaper articles about the sucrose work.[33]

Barker: Yes, there was some publicity that got out in the press.

##

Hughes: Do you remember why the name Program in Comparative Biochemistry was chosen? Why wasn't it just the Program in Biochemistry?

Barker: Well, there was a biochemistry department, you see, and we had to distinguish ourselves. It was in the medical school at the time. That was one of the slight complications. Also, Greenberg, the chairman of the medical school department, I think was a little sensitive about having another group with a name that was too similar. [laughs]

Hughes: What you were doing in your research was certainly comparative biochemistry, is that not true?

Barker: Well, we used various organisms, yes, mostly bacteria.

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[32] H. A. Barker, "Explorations of bacterial metabolism," *Annual Review of Biochemistry* 1975, 47:1-33.

[33] Barker's papers in the Bancroft Library are a rich source for all aspects of his career.

Hughes: But that wasn't really the agenda of the group as a whole?

Barker: Well, it wasn't actually a very big group. There were only about half a dozen people [faculty members], I think, who ever made use of this. It was mostly the students. I think my students, I think Hassid's students, and Chaikoff's students made use of this. And then some of the people in Food Technology--Joslyn, I think.

Teaching

Course in Soil Microbiology

Hughes: Do you remember what courses you taught?

Barker: I taught primarily two courses during this period. I was brought to the university to teach soil microbiology. Initially, when I first came, I think there were only two students that took the course. Dean Lipman had taught it several years in his spare moments, but he didn't do a very systematic job of it. He mostly came around and got people started on some simple experiments and perhaps once or twice during the semester gave a lecture or something. [laughs] It was a very informal course, and there were very few students. I think one reason I was hired was to take care of the students in Plant Nutrition that needed to know something about soil microorganisms. So after I came it was a required course in the soils curriculum.

Hughes: So you had a lot of students?

Barker: Well, not a lot because it wasn't a big group, but on the order of fifteen or twenty, or maybe it was as high as thirty sometimes.

The van Niel Approach to Biochemistry

Hughes: Were you more or less following van Niel's approach?

Barker: Well, to some extent, because that's what I knew. You see, I had studied with van Niel and I had been a year in Delft with Professor Kluyver in the laboratory where van Niel had originated. But this course in biochemistry was not primarily soil microbiology; it was only partially so. We used various sorts of material--plant material and perhaps some bacteria--to set up experiments that could be done rather easily.

Hughes: What was the second course you taught?

Barker: Well, some of the people in bacteriology also had been students of the same people in Stanford that I [had studied with], particularly van Niel at Pacific Grove, and so we had him in common.

Hughes: Was that Doudoroff?

Barker: Yes, Doudoroff, Stanier, and Ed Adelberg.

Hughes: Oh, Adelberg had been with van Niel, too?

Barker: Well, yes, I think he'd been there one summer.

Hughes: So they were all imbued with the van Niel approach to biochemistry?

Barker: I think so.

Hughes: What would you call it? Microbial biochemistry?

Barker: Yes. Ed Adelberg was a little more connected to genetics, but the others were primarily interested in biology and biochemistry.

Hughes: Stanier and Doudoroff were in Microbiology, is that correct?

Barker: They were in Bacteriology, primarily. K. F. Meyer was head of that department for many years.[34] And later on perhaps Stanier may have been head of the department for a short time--two years.

Hughes: Where was Adelberg?

Barker: He was in Bacteriology, too. He later on left and went somewhere in the East--to Yale, I think.

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[34] See the oral history with Karl F. Meyer in The Bancroft Library oral history series on public health at Berkeley.

Barker's Teaching Style

Hughes: Did you like teaching?

Barker: Oh, I don't think I was ever as enthusiastic about teaching as, for example, van Niel was, who was the person who really inspired me in the area of bacteriology.

Hughes: Could you describe your style of teaching?

Barker: Oh, I don't know. In the bacteriology course for agriculture students, I suppose to some degree I tried to imitate van Niel--not very well, however. But at any rate, we had a laboratory, and we had, I think, two lectures a week that more or less covered the general explanation of what we were trying to do.

Hughes: And you stood up and gave a formal lecture?

Barker: Yes. Well, it wasn't very formal. It was done in a different room; we had a regular lecture room. From time to time I would talk about the internal chemistry of microorganisms and so on. I would do that in the laboratory. The formal lecture part that was done in a lecture room generally covered the classical aspects of soil microbiology.

Hughes: What would you classify under classical? [laughter]

Barker: Well, I had studied with van Niel in Pacific Grove, and we learned a lot about the biochemical systems bacteria utilized. Then I was in Holland for a time, and I enjoyed lectures from Professor Kluyver that were in Dutch. For a time I had a little difficulty following the Dutch. It was all very nonmedical, talking about soil transport issues.

Hughes: And emphasizing the biochemical aspects of the microbial world?

Barker: Yes, well, why they're important, you see, is because they changed the chemistry.

Hughes: In medical school, I suspect that you'd get quite a different emphasis.

Barker: Oh, yes, they'd be talking about disease. I was not interested in that at all. I wouldn't cover it unless it was something that just happened to be related.

Hughes: Did it make a difference to you whether your students came from a microbiological outlook and background as opposed to a biochemical?

Barker: Well, the students that I had were almost entirely students in the soils curriculum and they had a fairly modest amount of chemistry. They must have had some biochemistry, [but] they weren't very high-powered. The more complex and scientific aspects of the chemical reactions in bacteria I got into with Ph.D. students.

Hughes: They presumably came to you because they also were interested in the biochemical aspects.

Barker: Yes, they came from various other departments. I guess most of my students actually got their degrees in agriculture.

Research on Anaerobic Bacteria

Hughes: Do you have a favorite piece of research?

Barker: Well, at one stage I'd been interested in the transformations of some of the anaerobic bacteria that I worked with, particularly *Clostridium kluyveri*, which I named after the professor I worked with in Holland.

Hughes: Oh, you named it!

Barker: Yes, I was the one to isolate it. It was isolated from the mud of a Dutch canal. [laughs] I published quite a number of papers dealing with various aspects of its biochemistry which was rather interesting. I worked on that off and on for a number of years and a couple of my students got Ph.D.s working in this area.

Hughes: Is there anything more you want to say?

Barker: I don't think so.

Hughes: Thank you.

Transcribed and Final Typed by Amelia Archer

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THE PRICELESS INGREDIENT IS THE HONOR AND INTEGRITY OF THE MAKER

ROLAND J. DAHL
VICE-PRESIDENT
DIRECTOR OF RESEARCH AND DEVELOPMENT

May 4, 1959

Professor H. A. Barker
Department of Agricultural Biochemistry
337 Biochemistry and Virus Building
University of California
Berkeley, California

Dear Professor Barker:

As a result of the interest of members of our technical staff, my attention has been called to your experimental program studying the coenzyme forms of the B_{12} group of vitamins. We are very interested and impressed with your findings on what may prove to be the natural form of these important nutritional factors. We are, of course, primarily interested in the therapeutic value of the B_{12} coenzymes and would like to inform you of our intention to apply for licenses under any patents you and your university will obtain.

May I assure you of the willingness of our laboratory group to supply you without obligation, in the near future as in the past, with any materials that will be useful to you in carrying out your program.

Very truly yours,

Roland J. Dahl

Roland J. Dahl

May 6, 1959

Mr. R. M. Underhill
240 Sproul Hall
Campus

Dear Mr. Underhill:

I am enclosing a letter from R. J. Dahl, Vice-President of Squibb and Sons, which indicates that Squibb is interested in obtaining a license for B_{12} coenzyme production under the patent for which I recently applied. Enclosed is a copy of my reply. I assume that you will handle any further business arrangements.

Squibb has been very helpful in providing us with materials useful in our research on the B_{12} coenzymes. No other group has been so helpful with the scientific aspects of our work nor so easy to deal with, so I would look with favor upon a license application by this Company.

Recently I had a telephone conversation with Dr. Otto Behrens of the Lilly Research Laboratories, and mentioned that I had applied for a patent covering the B_{12} coenzymes. He expressed interest and said he expected to contact you before long.

With best regards,

Sincerely yours,

HAB:a

H. A. Barker

Contributions of Pharmaceutical Companies

Lilly

1) Agreed to test therapeutic value of coenzymes & report results.
2) Provided biol. materials from which coenzymes may be isolated.
3. Supplied 1.5 g. of 5,6 dimethyl benzimidazole & various methods
4. Offered patent application assistance.

Merck

1. Supplied 1 g. dimethyl benzimidazole, 20 mg Factor B & 20 mg of hydroxo cobalamin.
2. Supplied 2 lbs. of bacterial cells (Clost. tetanom.) & promised more. Did not supply commercial source of coenzymes when requested
3. Supplied patent/application assistance (Mr. Bassford)
4. Offered to do biological testing.

Squibb

1. Supplied 6 Kg of bacterial cells (best coenzyme source available free)
2. Supplied 10 small batches of cells containing various coenzyme analogues.
3. Supplied various cultures, methods and data to assist our research activities.

October 20, 1959

Mr. Robert M. Underhill
Secretary and Treasurer of the Regents
University of California
615 University Hall
Campus

Dear Mr. Underhill: Re: Vitamin B_{12} Compounds

I was glad to receive copies of your letters to the various pharmaceutical companies indicating the present status of the negotiations concerning licensing under my patent application.

At present there is only a single publication dealing with coenzymes possessing vitamin B_{12} activity. This is a paper published in the Proceedings of the National Academy of Sciences (U.S.), 45, 521-525 (1959), a copy of which is enclosed. This paper describes some properties of the partially purified coenzymes, but does not describe the methods of purification nor the final isolation of the crystalline compounds. The first paper giving this information will be published in the Journal of Biological Chemistry, probably in January 1960. I expect that any further foreign patent applications should be made before this date.

I should mention that there is now considerable evidence that our "vitamin B_{12} coenzyme" is approximately as effective as vitamin B_{12} itself in the treatment of pernicious anemia and in the nutrition of chickens. We are still looking for situations in which the compound may be more effective than vitamin B_{12} as a nutrient or chemotherapeutic agent for animals or man. There is extensive interest in the coenzyme as a reagent for biochemical and medical research and there will certainly be some commercial market for these purposes at least.

Yours sincerely,

HAB:a

H. A. Barker

United States Patent Office

3,037,015
Patented May 29, 1962

3,037,015

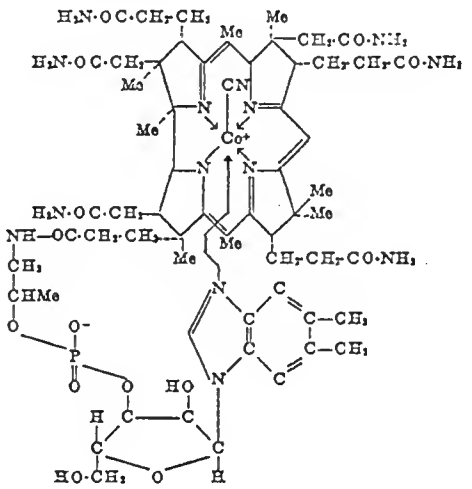
B_{12} COENZYMES AND PROCESSES FOR PREPARING THE SAME

Horace Albert Barker, Berkeley, Calif., assignor to The Regents of The University of California, Berkeley, Calif.

No Drawing. Filed Apr. 13, 1959, Ser. No. 805,728
16 Claims. (Cl. 260—211.5)

This invention is concerned generally with novel coenzymes having vitamin B_{12}-activity and with procedures for preparing them. More particularly, the invention relates to the new chemical compound, coenzyme B_{12}, and novel vitamin B_{12}-active analogs thereof, and to novel processes for producing these new coenzyme B_{12} compounds by controlled bacteriological synthesis and degradation of the resulting bacterial cell material. These novel vitamin B_{12}-active coenzyme B_{12} compounds, which can be characterized by their property of activating the enzymatic conversion of glutamate to mesaconate via B-methylaspartate, are valuable as feed supplements and for the treatment of nutritional diseases. They are further valuable as growth-promoting agents and in biological investigations of essential enzymatic reactions involved in metabolism and maintenance of health.

Vitamin B_{12}, which possesses marked and effective action in the therapeutic treatment of Addisonian pernicious anemia and other macrocytic anemias, may be chemically represented as follows:



Consistent with this structure, vitamin B_{12} and vitamin B_{12}-like compounds (which differ from vitamin B_{12} in that the cyano radical attached to the cobalt atom is replaced by a different grouping, and which are convertible to vitamin B_{12} per se by treatment with cyanide ion) are called cobalamins; vitamin B_{12} itself is referred to as cyanocobalamin; vitamin B_{12}-active compounds, in which the 5,6-dimethylbenzimidazole moiety present in the cobalamins (which acts as a bridge between the ribose and corphyrin portions of the molecule) is replaced by another nucleotide base, are herein referred to as vitamin B_{12} analogs.

The vitamin B_{12}-active coenzyme B_{12} compounds, subject of the present invention, are structurally similar to vitamin B_{12} and those of its cyano analogs, in which the nucleotide base is a benzimidazole compound (such as benzimidazole, 5,6-dimethylbenzimidazole and 5-hydroxybenzimidazole) but differ from these vitamins B_{12} compounds in lacking a cyano group and in possessing an adenine moiety attached to the corphyrin portion of the molecule. These novel vitamin B_{12}-active coenzyme B_{12} compounds as, for example, coenzyme B_{12} (which contains 5,6-dimethylbenzimidazole as the nucleotide base and which is convertible to vitamin B_{12} by treatment with cyanide ion), benzimidazole-coenzyme B_{12}, hydroxybenzimidazole-coenzyme B_{12}, and the like, are potent growth-promoting agents, and are valuble in nutrition and in the treatment of nutritional diseases. As the first coenzymes of the vitamin B_{12}-active group, these new coenzyme B_{12} compounds are particularly valuable to biochemical and medical research workers in connection with investigations of essential enzyme reactions involved in normal metabolism and maintenance of health, as well as in studies of abnormal metabolic processes characteristic of certain diseases. The vitamin B_{12}-active coenzyme B_{12} compounds can be characterized by their property of supporting the growth of *Ochromonas malhamensis* and by their ability to activate the enzymatic conversion of glutamate to mesaconate via β-methylaspartate.

These coenzyme B_{12} compounds are produced by fermenting, with a vitamin B_{12}-activity producing microorganism, an aqueous nutrient medium containing, where indicated, the benzimidazole precursor corresponding to the coenzyme B_{12} compound desired. As the vitamin B_{12}-activity producing organism, selected strains of microorganisms belonging to the Schizomycetes are ordinarily employed, particularly certain strains of the genus Streptomyces, the genus Bacillus, the genus Propionibacterium, the genus Alcaligenes, the genus Pseudomonis, the genus Mycobacterium, and the genus Clostridium, preferably strains selected from the species *Streptomyces griseus, Streptomyces albidoflavus, Streptomyces fradiae, Streptomyces venezuelae, Bacillus megaterium, Propionibacterium shermanii, Propionibacterium freudenreichii, Propionibacterium arabinosum, Alcaligenes faecalis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas lumichroma, Mycobacterium smegmatis, Clostridium tetanomorphum,* and the like. The benzimidazole precursor corresponding to the coenzyme B_{12} compound desired is ordinarily incorporated in the nutrient medium prior to fermentation, although many vitamin B_{12}-activity producing genera (for example Streptomyces, Bacillus, Propionibacterium, Pseudomonas and Mycobacterium) produce substantial yields of coenzyme B_{12} per se, utilizing nutrient mediums not containing added 5,6-dimethylbenzimidazole.

The vitamin B_{12}-activity producing microorganisms utilized in producing the new coenzyme B_{12} compounds are conveniently selected by testing their fermentation broths, using the protozoan *Ochromonas malhamensis* as the assay organism. A culture of the microorganism under investigation is diluted, plated out on a solid nutrient medium, and incubated to produce a considerable number of single-cell colonies. Individual colonies picked for inoculum development are separately grown in liquid nutrient mediums supplemented with cobalt nitrate at a concentration of 1 p.p.m. and with 5,6-dimethylbenzimidazole at a concentration of 0.0001 molar in suitable vessels and incubated either in presence or absence of oxygen, depending on the requirement of the organism. The fermentation broths are heated (where necessary) to coagulate the cells, and the resulting solution is assayed for *Ochromonas malhamensis* activity.

The basal medium employed for the growth of *Ochromonas malhamensis*, which eliminates non-specific growth stimulants present in certain crude extracts to which the

organism has proved susceptible, has the following composition:

Casein hydrolysate	g.	5
Glucose	g.	10
Diammonium hydrogen citrate	g.	0.8
KH_2PO_4	g.	0.3
$MgSO_4 \cdot 7H_2O$	g.	0.2
$CaCO_3$	g.	0.15
Ethylenediamine tetra-acetic acid	mg.	50
$MnSO_4 \cdot H_2O$	mg.	61.5
$ZnSo_4 \cdot 7H_2O$	mg.	110
$FeSO_4 \cdot 7H_2O$	mg.	10
$CoSO_4 \cdot 7H_2O$	mg.	3
$CuSo_4 \cdot 5H_2O$	mg.	0.4
H_3BO_3	mg.	0.6
KI	mg.	0.01
$Na_2MoO_4 \cdot 2H_2O$	mg.	50
DL-tryptophane	mg.	100
DL-methionine	mg.	200
L-cystine	mg.	100
Choline chloride	mg.	2
Inositol	mg.	10
p-Aminobenzoic acid	mg.	1
Thiamine	mg.	2
Biotin	μg.	10
Tween 80 [1]	mg.	1

pH adjusted to 5.5.
Distilled water to 200 ml.

[1] A polyoxyethylene derivative of sorbitol mono-oleate suitable for use in microbiological cultures.

The test organism is maintained in the basal medium diluted 1 part of medium to 5 parts distilled water and supplemented with 0.2 mμg. cyanocobalamin/ml. The diluted medium is dispensed in 10 ml. amounts into 50 ml. conical flasks, which are then plugged and sterilized by autoclaving for 15 minutes at 10 lb./in.² pressure. The organism is transferred in this medium at 5-day intervals, and incubated in a cabinet at approximately 27° C., 1 ft. below a 60 w. tungsten filament lamp. After 5 days' incubation under these conditions the cell population density in the cultures reaches approximately 5,000,000 cells/ml. For inoculum, a 5-day culture is diluted 1:10 with sterile basal medium diluted 1:5, and 0.5 ml. is added to each assay tube.

Assays are set up in 19 x 150 mm. optical matched Pyrex test tubes. A standard solution of cyanocobalamin containing 0.2 mμg./ml. is added to paired tubes at levels of 0.25, 0.5, 1.0, 2.0 and 4.0 ml. Test extracts of fermented broth are added to paired tubes at the same levels (following a preliminary experiment to determine whether the broth has any *Ochromonas malhamensis* activity and the approximate value of this activity), and water is added to the tubes to bring their fluid content to 4 ml. To each of the tubes is then added 1 ml. of the undiluted basal medium, the tubes are plugged with cotton, and autoclaved for 10 min. at 10 lb./in.² pressure. The tubes are then cooled, inoculated with 0.5 ml. of the 5-day diluted culture referred to hereinabove, placed in a shaking machine in an incubator at 29° C. and shaken in darkness for 72 hours. The tubes are then autoclaved, 5 ml. water are added to each, and the growth in each tube is determined turbidimetrically in a Klett-Summerson colorimeter using a 540 millimicron filter.

Since the size of the inoculum is constant for each tube, the growths obtained in the control and test cultures are proportional to the concentration of vitamin B_{12}-active substances contained therein. Comparison of the growth of the test culture with that in the controls gives a quantitative measure of the concentration of vitamin B_{12}-active substances (expressed as mμg. of cyanocobalamin/ml.) in the test cultures and, by a simple calculation, the precise content of vitamin B_{12}-active substances in the fermented broth taken from the original fermentation vessels.

From the above test, it is possible to determine whether a given microorganism is potentially capable of synthesizing vitamin B_{12}-active coenzyme B_{12} compounds, as well as the amount of vitamin B_{12}-active substances contained in the cells and fermentation broth obtained when said microorganism is used to ferment an aqueous nutrient medium.

The bacteriological production of the presently-invented coenzyme B_{12} compounds is conducted utilizing aqueous nutrient mediums ordinarily employed in the propagation of microorganisms. The usual nutrients include an energy source, a carbon source, a nitrogen source, inorganic salts, and growth factors when required. It is preferred to supplement the medium with a source of cobalt, such as cobalt nitrate; in addition, the appropriate benzimidazole precursor (e.g., benzimidazole; 5-hydroxybenzimidazole; 5,6-dimethylbenzimidazole) is ordinarily incorporated in the medium, although no added precursor is required for producing coenzyme B_{12} per se using many vitamin B_{12}-activity producing genera, as noted hereinabove. The carbon and energy can be provided by a carbohydrate such as dextrose, maltose, xylose, invert sugar, corn syrup, and the like, and by amino acids such as glutamic acid (in the form of its neutral salts). The nitrogen can be provided by an ammonium salt, amino acids, proteins or protein degradation products, obtained from proteins such as soy beans, oats, yeast, yeast extracts, casein, meat, blood meal, protein meat and bone scrap, salmon meal, fish meals, fish solubles, distillers solubles, and the like. If desired, the microorganisms can be propagated using proteins or protein degradation products without any carbohydrate being present in the medium, in which case the proteins serve as the source of energy, carbon and nitrogen required by the microorganism.

The aqueous nutrient medium is sterilized and inoculated with a culture of the selected microorganism strain, and the mixture is incubated under conditions appropriate to the particular microorganism employed. Since the coenzyme B_{12} compounds are extremely sensitive to decomposition on exposure to visible light, all operations involved in the production of these compounds are conducted in the substantial absence of light. It may be noted that coenzyme B_{12} compounds are not obtained in accordance with the methods utilized heretofore for obtaining vitamin B_{12} compounds, since those methods not only failed to provide effective protection from light, but also conventionally involved treatment with cyanide ion and/or acidification to pH 3, thus precluding the preparation of the coenzyme which is highly unstable in the presence of cyanide or acid. The fermentation is allowed to proceed for a time sufficient for the bacterial cells to reach maximum growth, at which time the fermented mixture is centrifuged or filtered, the supernatant solution is discarded, the cellular material is recovered as a paste and subjected to degradation to produce the coenzyme B_{12} compound. Alternatively the fermented mixture is heated or allowed to undergo lysis, thereby producing a solution of the coenzyme B_{12} compound in the fermentation broth; avoidance of cyanide and/or acid, and protection from light are essential in this operation as well as in all subsequent treatments if the coenzyme B_{12} compound is to be obtained. The former method, where the cells are separated from the fermented mixture and then subjected to degradation, results in the production of a relatively concentrated aqueous solution of the coenzyme B_{12} compound which is substantially free from impurities present in the original broth; the latter method, which produces a relatively dilute and impure solution of the coenzyme in the whole broth, has the advantage of avoiding the difficult separation of the cellular material from the broth.

The degradation of the cellular material (where the cell paste is separated from the broth) is conveniently conducted by heating the diluted aqueous cell paste preferably at a temperature within the range of approximately 70–100° C., although higher or lower temperatures may

be employed if desired; the heating is continued for a time sufficient to coagulate the cellular material, e.g., about 2 to 20 minutes at 100° C. Alternatively, the cells are subjected to the action of an alcoholic solution as, for example, a solution of a lower alkanol such as methanol, ethanol, propanol, and the like, having a concentration in water within the range of approximately 70–100%. It is ordinarily preferred to mix the aqueous cell paste separated from the fermented mixture with enough ethanol to give a final ethanol concentration of about 80%. Irrespective of the method utilized in coagulating and precipitating the cellular material, there is obtained, following separation of precipitated cells, a solution of the coenzyme B_{12} compound; this solution (where alcohol is present) is then subjected to distillation in vacuo, thereby evaporating the alcohol. The aqueous solution of the coenzyme is then passed through a cation exchange resin (preferably a sulfonic acid type resin such as Dowex-50, 8x, manufactured by the Dow Chemical Co.) in the sodium form, thereby absorbing cationic substances from the solution; the eluate is then passed through an anion exchange resin containing quaternary ammonium groupings (such as the Dowex-2, 8x, resin manufactured by the Dow Chemical Co.) in the hydroxide or acetate form, thereby absorbing anionic substances including acidic nucleotides and amino acids. The resulting eluates and washings are combined and adjusted to pH 6.5–7.0, conveniently with 1 N acetic acid solution.

The resulting solution is then extracted with an hydroxylated organic solvent characterized as being partially immiscible with water as, for example, a phenolic solvent such as phenol or cresol, an alkanol such as butanol, amyl alcohol, and the like, or a mixture of such hydroxylated solvent and a hydrophobic solvent, as for example a hydrocarbon solvent such as benzene, toluene, a chlorinated hydrocarbon solvent such as ethylenedichloride, trichlorethylene, and the like. There is added to the hydroxylated organic solvent extract a lower ketone such as acetone, methylethyl ketone, and/or a dialkyl ether such as ethyl ether, dipropyl ether, and the like, thereby forming an upper organic layer containing the hydroxylated organic solvent and a lower aqueous phase containing the coenzyme. It is ordinarily preferred to utilize phenol as the hydroxylated organic extracting solvent and to add to the phenolic extract a 1:3 mixture of acetone-ether. The organic layer is extracted with water until the aqueous extract is virtually colorless. The combined aqueous extracts are washed with a substantially water-immiscible solvent such as ether to remove hydroxylated organic solvent, and the aqueous layer is distilled in vacuo, thereby evaporating ether remaining in the aqueous phase and forming a relatively concentrated aqueous solution of the coenzyme B_{12} compound.

This solution, the color of which (depending on the concentration of the coenzyme B_{12} compound) varies between orange and red, is then passed into a column of a weakly acidified (pH approximately 3) cation exchange resin, preferably of the sulfonic acid type, in the mixed sodium-hydrogen form; as the resin, it is preferred to use a copolymer of styrene in which the divinylbenzene component is between 1 and 4%. (A commercially-available resin of this type is manufactured by the Dow Chemical Co. under the trade name Dowex 50W–2x.) Resins containing higher cross-linking have proved less satisfactory. The resin is adjusted to pH approximately 2.5 to 3.5 (mixed Na^+—H^+ form) at which pH coenzyme B_{12} compounds have a positive charge and are adsorbed on the resin; the free vitamin B_{12} compounds in the cyano form (which are substantially neutral) are not appreciably adsorbed at this pH. The solution of coenzyme B_{12} compound is rinsed into the column and the coenzyme is adsorbed to form a thin orange-red band at the top of the column. (All observations as to color of solutions and of bands of material adsorbed on columns are made with dim light and with minimal time of exposure to avoid substantial decomposition of the coenzyme B_{12} compound.)

The resin column is then subjected to differential elution with buffer solutions of gradually decreasing acidity containing Na^+ as the competing ion. It is ordinarily preferred to employ solutions of sodium acetate within the range 0.01 to 0.2 molar, although other sodium buffer salts, e.g., sodium propionate, sodium phosphate, and the like, may be used if desired. The initial elution is preferably conducted with 0.03 M sodium acetate at pH 4.6 to 4.8; when about 5–6 column volumes of eluate have been collected, the eluting solution is changed to 0.03 M sodium acetate at pH 5.4 to 5.6 and an additional 10 column volumes of eluate is collected. (The properties of the coenzyme B_{12} compounds are such that they are not appreciably eluted under the above conditions, and the initial elution at pH 4.6 to 4.8 can be omitted if desired since impurities eluted at pH 4.8 are also eluted at pH 5.6.) The column is then eluted with 0.03 M sodium acetate at pH 6.0–7.2 whereby the coenzyme B_{12} compound is substantially completely eluted from the column.

The primary property described herein for selection of those fractions of the eluate which contain the coenzyme B_{12} compounds is the property of these compounds of absorbing light in the region of 260 mu, at which wave length coenzyme B_{12} compounds have their maximum absorbance. Thus, the absorbance values of the individual eluate fractions collected from the chromatogram are measured and plotted as a function of the fraction number. On such a plot, each particular compound elutes at maximum concentration in a particular tube, and the concentration and the absorbance of the earlier and later tubes are progressively lower; this necessarily results in an absorbance "peak." The absorbance peaks observed in this procedure may correspond to fractions which are colorless or variously colored. Only those peak fractions (using the 260 mu wave length) which are orange or red in color contain coenzyme B_{12} compounds in substantial concentrations.

The further selection of the fractions containing coenzyme B_{12} compounds from amongst the red or orange colored peak fractions is based upon the determination of coenzyme activity using the glutamate-mesaconate spectrophotometric coenzyme assay and upon the determination of the entire ultra violet and visible absorption spectrum of the fractions.

The glutamate-mesaconate spectrophotometric coenzyme assay is based on the observation that the rate of formation of mesaconate from glutamate by suitable enzyme preparations of *Clostridium tetanomorphum* strain H1 is dependent upon the concentration of coenzyme B_{12} compounds in the reaction mixture. Under suitable conditions, defined below, the rate of mesaconate formation in the assay system is substantially proportional to the concentration of a particular coenzyme B_{12} compound.

The assay depends upon the following chemical reactions catalyzed by suitable enzyme containing extracts of *C. tetanomorphum*:

$$\text{Glutamate} \xrightarrow[\text{I}]{} \beta\text{-methylaspartate} \xrightarrow[\text{II}]{} \text{mesaconate} + NH_3$$

The formation of mesaconate is detected by an increase in ultra violet light absorption at wave lengths below 300 mu. A wave length of 240 mu is convenient to use for this purpose. The rate of mesaconate formation is measured by the rate of change of absorbance at 240 mu, determined with a spectrophotometer with silica cells capable of measuring absorbance at wave lengths below 300 mu. The absorbance is expressed in units which equal the log of the incident light intensity divided by the intensity of the transmitted light.

Extracts containing enzyme suitable for the assay are

prepared as follows: 15 g. of cell paste of *C. tetanomorphum* strain H1, freshly harvested from a 0.1 M glutamate —0.3% yeast extract medium, are suspended in 30 ml. of 0.02 M potassium phosphate buffer pH 7.6 containing 0.07 M-mercaptoethanol. All subsequent operations in the preparation of the enzyme extract are carried out at 0–5° C. Approximately 3 g. of grade FFF corundum powder and 5 g. of moist, acid-washed activated charcoal Nuchar are added and the suspension is exposed to sonic vibration in a Raytheon 10 kc. sonic oscillator for 10 minutes at 0–5°, thereby disrupting the cells. The suspension is then centrifuged for 10 minutes at 16,000×*g* and the sediment is discarded. To 34 ml. of the supernatant solution, 20 ml. of 1% (w./v.) protamine sulfate (Nutritional Bioch. Corp.) is added slowly with mechanical stirring, in order to remove nucleic acids. After stirring for 5 minutes the precipitate is removed by centrifugation at 16,000×*g*. Small aliquots of the clear supernatant solution, containing 20 to 25 mg. of protein per ml., are placed in small plastic tubes and immediately frozen. When stored at —10° C., the enzyme system retains much of its activity for several months. Repeated thawing and freezing of the enzyme solution and storage at 0° C. results in rapid loss of activity. For this reason the enzyme solution is divided, before being frozen, into small aliquots sufficient for the assays to be performed each day.

When the enzyme extract is prepared in the absence of charcoal, the resulting extract catalyzes both Reactions I and II. However, when the extract has been treated with a suitable charcoal adsorbent either during or after the breaking of the cells, the coenzyme B_{12} compounds normally present in such extracts are adsorbed by the charcoal and thus removed from the extract. Such charcoal-treated extracts catalyze Reaction II, but they cannot catalyze Reaction I at a significant rate unless some coenzyme B_{12} compound is added.

The reaction mixture contains per ml., 0.01 M monosodium L-glutamate, 0.05 M tris(hydroxymethyl)aminomethane chloride buffer pH 8.02, 0.01 M KCl, 0.001 M $MgCl_2$, 0.05 ml. of a charcoal- and protamine-treated enzyme preparation (see above) and sufficient coenzyme to give an absorbance change of 0.01 to 0.08 unit per minute at 240 mu corresponding to the formation of 0.0026 to 0.021 μmole of mesaconate per minute. The reaction is started by the addition of enzyme and readings are taken at 0.5 minute intervals for three minutes. The rate of reaction is calculated from the change in absorbance during the last two minutes. The reference cell contains sufficient mesaconate, usually about 4×10^{-4} M, so that the absorbance of the reaction mixture falls between 0 and 0.5.

The rate of mesaconate formation as measured by the rate of absorbance change under the assay conditions is approximately proportional to the concentration of coenzyme B_{12} compound over the limited range indicated above. The corresponding range of concentrations of the coenzyme B_{12} compound differs with different forms of the coenzyme. With coenzyme B_{12} per se the useful range is approximately 2×10^{-7} M to 2×10^{-6} M, whereas for benzimidazole-coenzyme B_{12} (whose coenzyme activity is about 60 times that of coenzyme B_{12}) it is approximately 4×10^{-9} M to 4×10^{-8} M.

The coenzyme activity in the glutamate-mesaconate spectrophotometric coenzyme assay is expressed in units of absorbance change per minute under the assay conditions. One activity unit is the amount of coenzyme that causes an absorbance change of one absorbance unit per minute. The activity unit does not have an absolute value because the activity of the charcoal-treated enzyme extract differs from one preparation to another. Therefore the activity unit has a relative value which is determined in relation to the activity of a standard sample of coenzyme under identical assay conditions. A convenient reference standard is a solution of a purified sample of the benzimidazole-coenzyme B_{12} of known concentration, although any coenzyme sample of known concentration can be used as the standard, if desired. The relative activity of the unknown sample is determined with respect to the reference sample by direct comparison in this glutamate-mesaconate spectrophotometric coenzyme assay.

The glutamate-mesaconate spectrophotometric coenzyme assay is employed not only for determining coenzyme B_{12} compounds in fractions from the chromatogram, but is also used to assay for coenzyme B_{12} compound-activity at various stages in the purification including direct extracts of disrupted cell paste. The determination of coenzyme B_{12} compound-activity in such cell paste extracts provides a convenient method for selecting microorganisms suitable for coenzyme preparation. A convenient method for determining extractable coenzyme activity in disrupted microorganism cell paste is to suspend 50 mg. of the cell paste in 1 ml. of 0.01 M sodium acetate buffer, pH 6.0 and heating the suspension in a boiling water bath for 5 minutes. The mixture is rapidly cooled to 0° C. and centrifuged for 5 minutes at 16,000×*g* and aliquot of the color supernatant solution is then assayed in the glutamate-mesaconate spectrophotometric coenzyme assay.

In unfractionated extracts of microbial cells, compounds are sometimes present that cause a non-specific absorbance change in the glutamate-mesaconate spectrophotometric coenzyme assay. The presence of such compounds may be detected and a suitable correction found by doing a control assay in which glutamate is omitted from the assay mixture. An additional correction should also be made for the small absorbance change that sometimes occurs in the absence of added coenzyme.

As noted hereinabove, this glutamate-mesaconate spectro-photometric coenzyme assay facilitates the selection of those eluate fractions from chromatogram which contain coenzyme B_{12} compounds. The further selection of eluate fractions containing coenzyme B_{12} compounds in a state of high purity is achieved by determining the apparent specific-coenzyme-activity of the active fractions. By "specific-coenzyme-activity" is meant the ratio of the activity as determined on a particular sample divided by the absorbance of that sample at 260 mu. Since the absorbance at 260 mu is determined by certain impurities as well as by concentration of coenzyme B_{12} compounds, the ratio of activity to absorbance (specific-coenzyme-activity) gives a quantitative measure of the purity of the coenzyme B_{12} contained in the fraction; thus, fractions having a constant value for specific-coenzyme-activity can be assumed to be free of inactive impurities absorbing at 260 mu.

The specific coenzyme activity provides a convenient method for characterizing coenzyme B_{12} compounds and is particularly effective for distinguishing between the benzimidazole coenzyme B_{12} and coenzyme B_{12}, since the specific activity of the former is approximately 60 times that of the latter.

The fractions are further characterized by determination of the ultra violet and visible absorption spectrum in order to establish whether the coenzyme B_{12} compound (demonstrated in the previous tests to be present in such fractions in relatively pure form) is the desired coenzyme B_{12} compound. The spectra of the coenzyme B_{12} compounds are similar in lacking the prominent absorbance peak in the 350–367 mu region which is characteristic of all previously known vitamin B_{12} compounds. While the spectra of the coenzyme B_{12} compounds are generally similar, they can readily be distinguished from one another. For example, the adenine-coenzyme B_{12} differs markedly from the benzimidazole-coenzyme B_{12} compounds in having a prominent absorbance maximum at 458 mu, whereas the benzimidazole-coenzyme B_{12} compounds have a comparable absorbance maximum at

about 520 mu. Also, the spectrum of benzimidazole-coenzyme B_{12} has an inflection at 280 mu, whereas the spectrum of coenzyme B_{12} (containing 5,6-dimethylbenzimidazole) has an inflection at 287 mu.

The homogeneous fractions from the column containing a pure coenzyme B_{12} compound are now combined, desalted by extraction into a phenolic solvent and reextracted back into water to give a salt-free concentrated aqueous solution of the pure coenzyme B_{12} compound. Such a solution may be used in the preparation of the crystalline coenzyme either by slow evaporation or by addition of acetone or other organic solvent in which the coenzyme B_{12} compound is relatively insoluble. Alternatively, this solution can be used directly as a substantially pure form of the coenzyme B_{12} compound for nutritional purposes or for metabolic studies.

In accordance with the foregoing procedure, and utilizing vitamin B_{12}-activity producing Schizomycetes in conjunction with a benzimidazole compound as precursor, there are obtained vitamin B_{12}-active coenzyme B_{12} compounds containing a benzimidazole or similar nucleus, as for example coenzyme B_{12} (which contains the 5,6-dimethylbenzimidazole) benzimidazole-coenzyme B_{12}, 5-hydroxy-benzimidazole-coenzyme B_{12}, 5-amino-benzimidazole-coenzyme B_{12}, 5-nitro-benzimidazole-coenzyme B_{12}, 5-methyl-benzimidazole-coenzyme B_{12}, and the like. Other vitamin B_{12}-active coenzyme B_{12} compounds containing heterocyclic compounds other than the benzimidazoles attached to the ribose may be likewise produced utilizing vitamin B_{12}-activity producing Schizomycetes in conjunction with the appropriate heterocyclic compound as precursor. These vitamin B_{12}-active coenzyme B_{12} compounds may be administered for their nutritional effect as such or in the form of their solutions in pharmacologically accepted liquid diluents, such as water, or in admixture with pharmaceutical carriers or as feed supplements in admixture with pharmacologically accepted feed additives, and the like.

The coenzyme B_{12} compounds produced in accordance with this invention differ from the vitamin B_{12} compounds by containing an adenine moiety in addition to the heterocyclic base attached to ribose in the vitamin B_{12} compounds. The spectra of these coenzyme B_{12} compounds differ greatly from the spectra of the corresponding B_{12} vitamins by having a peak with the highest extinction coefficient at approximately 260 mu, and by lacking the prominent peak with a high extinction coefficient in the 350–367 mu region which is characteristic of all of the previously known vitamin B_{12} compounds. The coenzyme B_{12} compounds are readily decomposed by exposure to visible light or by exposure to cyanide ion. Either of these treatments results in progressive and finally complete loss of coenzyme activity. Either exposure to light or to cyanide ion causes removal of adenine or an adenine derivative from the coenzyme.

A comparison of some properties and certain structural features of coenzyme B_{12}, benzimidazole coenzyme B_{12}, and vitamin B_{12} are set forth in the following table:

	Coenzyme B_{12}		Benzimidazole-Coenzyme B_{12}		Vitamin B_{12}	
	Max. mu	$E^{1\,cm}_{1\%}$	Max. mu	$E^{1\,cm}_{1\%}$	Max. mu	$E^{1\,cm}_{1\%}$
Absorption Spectrum in 0.03M NaAc pH 6.7	260	35.5	261	35.5	278	16.0
	315	12.8	303–305	12.8		
	336–337	12.5	316	12.8		
					361	27.8
	375	9.9	375	9.9		
	523	7.5	520	7.5		
					550	8.6
Inflections	287		280			
	440–445		440–445			
	500		500			
	Moles per Atom of Cobalt					
Components:						
Benzimidazole	0		1		0	
5,6-Dimethyl-benzimidazole	1		0		1	
Adenine	1		1		0	
Ribose	1		1		1	
Phosphate	1		1		1	
Cyanide	0		0		1	
Charge at pH 4.8	+		+		0.	
Molecular Weight	1,600–1,700		1,600–1,700		1,355.	
Coenzyme Activity	1.7		100		None.	
Ochromonas Malhamensis Activity	+		+		+.	
Stability in Cyanide Solution.	Converted to dicyanocobalamin. Coenzyme activity lost. Adenine removed.		Converted to dicyanobenzimidazole cobamide. Coenzyme activity lost. Adenine removed.		Converted to dicyano form.	
Stability in Light	Converted to hydroxocobalamin, or very similar cpd. Coenzyme activity lost. Adenine removed (as derivative).		Converted to hydroxobenzimidazole cobamide, or very similar cpd. Coenzyme activity lost. Adenine removed (as derivative).		CN slowly lost.	

EXAMPLE 1

A culture medium for the production of the benzimidazole-coenzyme B_{12} is prepared as follows: A sterile 20 liter Pyrex bottle is filled with 14 liters of distilled water at 35–37° C. To this are added 4 liters of sterile solution A and 700 ml. of sterile solution B, the compositions of which are described hereinbelow, 200 ml. of sterile 4 M glucose and 200 ml. of 10^{-2} M benzimidazole. The bottle is then rotated to mix the contents, 0.6 g. of dry (non-sterile) sodium hydrosulfite ($Na_2S_2O_4$) is added, and the contents again mixed.

Sterile Solution A.—Preparation and Sterilization.

Basamin (Anheuser Busch yeast extract)	g..	500
Accent (monosodium glutamate)	g..	2700
$MgSO_4$, 2 M	ml..	80
$FeSO_4$, 0.2 M	ml..	32
$MnCl_2$, 0.1 M	ml..	16
Na_2MoO_4, 0.1 M	ml..	16
$CoCl_2$, 0.1 M	ml..	32
$CuCl_2$, 1 M	ml..	16

Distilled water to 32 liters.

To sterilize, place 4 liters of this solution in a 6-liter flask and sterilize for 45 minutes at 18 lb. steam pressure.

Sterile Solution B.—Preparation and Sterilization

KH_2PO_4 (reagent grade)	g..	170
$K_2HPO_4 \cdot 3H_2O$ (reagent grade)	g..	1,200

Distilled water to 5.6 liters.

To sterilize, place 700 ml. of this solution in a liter flask and sterilize for 45 minutes at 18 lb. steam pressure.

This medium is then inoculated with 750 ml. of an actively fermenting pure culture of a vitamin B_{12}- activity producing strain of *Clostridium tetanomorphum* (strain H1 which produces vitamin B_{12} active compounds when grown in a medium containing 5,6-dimethylbenzimidazole) prepared by inoculating 5 ml. of a semisolid agar (0.2%) culture of the bacteria into solution A supplemented with 0.05% of cysteine·HCl neutralized to pH 7 and incubating this for 18–24 hours at 37° C. The 20-liter bottle is then filled with distilled water and incubated at 35–37° C. until the culture reaches maximum turbidity indicating maximum growth. This usually requires from 15–20 hours, depending on temperature and condition of the inoculum, and corresponds to a reading of 20 to 25 ($2-\log g=0.6$ to 0.7) on an Evelyn colorimeter using 18.0 mm. O.D. tubes and a 540 mu (green) filter after correcting for the absorbance of the uninoculated medium.

The bacteria are then harvested by centrifugation at $20{,}000 \times g$. The 3–4 g. of cell paste per liter of medium thus obtained were placed in a wide mouthed polyethylene container, immediately frozen in a Dry Ice-alcohol mixture and stored at or below −10° C. until the isolation-purification procedure could be performed. The foregoing operation is carried out repeatedly to produce a total of 3.86 kg. of cell paste.

An 83% ethanol extract is prepared from 1 kg. of cells at a time. Two liters of absolute ethanol are added to 1 kg. of thawed cell paste and the mixture is homogenized for 30 seconds in a large Waring Blendor. The resulting suspension is poured into 2 liters of boiling 95% ethanol. The mixture is heated to boiling, allowed to stand at this temperature for 15 minutes and then filtered while hot through a layer of diatomaceous silica on 2 large Büchner funnels. The residue on the filter is sucked dry and resuspended in 2 liters of 80% ethanol. The suspension is filtered as before. The combined filtrates from 3.86 kg. of cells are combined and concentrated in vacuo to about 800 ml. To remove residual ethanol, 1.5 liters of distilled water is added and the solution is again concentrated to about 1 liter.

The resulting solution is turbid and a slimy precipitate forms after the solution is frozen and thawed. To remove the precipitate, which tends to clog the ion exchange resin columns subsequently used, 10 ml. of 1 M $ZnSO_4$ and 20 ml. of 1 N NaOH are added per liter of solution and after standing 5 minutes the resulting precipitate is removed by filtration through diatomaceous silica.

The clear solution is adjusted to pH 7 and passed by gravity flow through a 15 cm. high x 3.5 cm. diameter column of a resin copolymer of styrene and divinyl benzene containing sulfonic acid groups, 12% cross-linked, in the sodium ion form (Dowex 50, 50–100 mesh; Dow Chemical Company). The column is washed and sucked dry to recover the coenzyme completely. The final volume is about 2 liters.

The solution is adjusted to pH 8.5 with 2 N NaOH and passed by gravity flow through a 15 cm. high x 3.5 cm. diameter column of an anion-exchange resin containing quaternary ammonium groups, 8% cross-linked in the hydroxide ion form (Dowex-2, 50–100 mesh; Dow Chemical Company). This requires about 3 hours. The column is washed with water and the combined effluents pH 9.7 are neutralized with 90 ml. of 1 M acetic acid to pH 6.3. The volume is approximately 2.2 liters.

Phenol extraction.—Each liter of solution is extracted with 120 ml. of 90% (w./v.) phenol-water, then twice with 40 ml. of phenol-water. The phases are separated by centrifugation. The phenol phase (120 ml.) is washed twice with 20 ml. water. The wash water is reextracted with 4 ml. phenol, the water is discarded and the phenol extracts are combined. To 125 ml. of phenol phases are added 375 ml. of ether, 125 ml. of acetone and 10 ml. of water. The mixture is shaken and centrifuged to separate the aqueous phase. The organic phase is reextracted twice with 10 ml. of water. The combined aqueous phase from 2.2 liters of Dowex-2 treated solution is extracted three times with 5 ml. of ether to remove phenol and is aerated with nitrogen to remove ether.

Chromatographic purification.—The aqueous solution from the phenol extraction operation (volume approximately 70 ml.) containing the partially purified benzimidazole-coenzyme B_{12}, is acidified to pH 3.2 with 7 ml. of 1 N aqueous hydrochloric acid solution. Since the calculated salt concentration is about 0.09 M, the solution is diluted 5-fold to give a final salt concentration below 0.02 M; lower salt concentrations favor adsorption of the coenzyme on the resin. The acidified and diluted solution is passed into a resin column prepared as follows: 2 M sodium phosphate-phosphoric acid buffer pH 2.5 is allowed to pass under gravity through a column of a sulfonic acid type cation-exchange resin which is a copolymer of styrene and divinylbenzimidazole containing free sulfonic acid groupings, 2% cross-linked, 200–400 mesh (Dowex-50W, 200–400 mesh; 2% cross-linked; Dow Chemical Co.), which is initially in the acid form, until the effluent has the same pH as the added buffer. The resin is then washed with distilled water until the effluent is free from phosphate. The washed resin, in the mixed sodium ion-hydrogen ion form, is used to make a 1 cm. diameter x 80 cm. high column. During passage into the column, the benzimidazole-coenzyme B_{12} is adsorbed on the resin.

The column is then eluted with approximately 800 ml. of 0.03 M sodium acetate at pH 5.2 followed by about 1500 ml. of 0.03 M sodium acetate pH 6.2; individual 16 ml. fractions are collected throughout the elution and their absorbance at 261 mu measured. The elution of the benzimidazole-coenzyme B_{12} which begins after approximately 400 ml. of the pH 6.2 buffer has passed through the column, and is completed when approximately 750 ml. of this buffer has passed through the column, is recognized by the appearance of an intensely reddish-orange color and by the appearance of a prominent and rather symmetrical 261 mu absorbance peak. The homogeneity of the coenzyme material eluted in the peak fractions is determined by comparing the coenzyme activity, as measured by the glutamate-mesaconate spectrophotometric coenzyme assay, with the absorbance of the various fractions; this is expressed as relative specific activity and is substantially constant for the central portion of the peak containing 80–90% of the total absorbance.

The peak fractions, which have (as noted) essentially constant specific activity, and which possess the type of spectrum characteristic of the coenzyme B_{12} compound containing benzimidazole attached to ribose, are combined to give a total volume of approximately 280 ml. This solution contains approximately 53 umoles of benzimidazole-coenzyme B_{12} (as determined by absorbance

measurements at 261 mu and the estimated molar extinction coefficient of 35.5×10^6 cm.²/mole at 261 mu), and in substantially pure form as indicated by the absorption spectrum.

The solution containing the benzimidazole-coenzyme B_{12} and buffer salt is saturated with phenol and is extracted three times with 0.15 volume of 90% aqueous phenol. The combined phenol phase is washed twice with 0.05 volume of water (which is discarded), and then is diluted with 3 volumes of ethyl ether followed by 1 volume of acetone. The phenol-ether-acetone solution is extracted three times with 0.1 volume of water. The combined aqueous solution containing the coenzyme is extracted three times with ether to remove residual phenol and is then coacentrated in vacuo to remove residual ether. The deep red aqueous solution, containing approximately 48 umoles of coenzyme in a volume of 8 ml. is placed in a vacuum desiccator over concentrated sulfuric acid as a desiccant. The desiccator is evacuated and the solution is allowed to concentrate at 3° C. to a volume of approximately 2 ml. during a period of several days. During concentration of the solution, crystallization of the coenzyme occurs. The mother liquor is decanted, the crystals adhering to the walls of the container are washed first with 90% acetone, then with 100% acetone, and finally with ethyl ether. After removal of ether in a vacuum desiccator there is obtained approximately 50–70 mg. of substantially pure crystalline benzimidazole-coenzyme B_{12} in hydrated form.

The benzimidazole-coenzyme B_{12} forms prismatic crystals having some diamond-shaped and some rectangular faces. The crystals are conspicuously dichroic, being either light yellow or deep red or a mixture of these colors. The diamond-shaped faces appear either yellow or red, depending on the angle of observation; the rectangular faces appear red. The absorption spectrum of an aqueous solution of this crystalline benzimidazole-coenzyme B_{12} is substantially identical with the absorption spectrum of the peak column fractions from which the crystalline material is obtained. The specific coenzyme-activity of the dissolved crystalline benzimidazole-coenzyme B_{12} is likewise substantially the same as that of the peak column fractions.

The ultra violet and visible absorption spectrum of crystalline benzimidazole-coenzyme B_{12} hydrate (prepared in accordance with the foregoing procedure) dissolved in water showed maxima at 261, 315, 375, and 250 mu and the corresponding

$$E_1^{1\%}_{1\text{ cm.}}$$

values were 175, 68, 53, and 39; the spectrum had inflection points at 280, 440–445, and 500 mu. The cobalt content of this crystalline benzimidazole-coenzyme B_{12} hydrate was found to be 3.1% corresponding to an apparent molecular weight of approximately 1900. This value includes an unknown amount of water of crystallization; the molecular weight of the anhydrous crystalline coenzyme, calculated on the basis of 1 mole of cobalt, is estimated to be within the range 1500–1700. Analysis of the benzimidazole-coenzyme B_{12} showed that it contains per mole of cobalt approximately one mole each of benzimidazole, adenine, ribose and phosphate.

Treatment of the benzimidazole-coenzyme B_{12} with 0.1 M KCN for approximately 30 minutes at room temperature results in complete loss of coenzyme activity and results in the formation of a compound which in the region between 300 mu and 650 mu has an absorption substantially identical with that of the dicyano form of vitamin B_{12} with absorption peaks at 304 mu, 367 mu, 416 mu, 540 mu, and 579 mu.

Exposure of the benzimidazole-coenzyme B_{12} to visible light, such as a 100 w. tungsten filament lamp at a distance of 1 foot for a period of 30 minutes results in virtually complete loss of coenzyme activity and results in the formation of a compound having an absorption spectrum very similar to that of hydroxocobalamin in the region between 320 mu and 600 mu and showing maxima at 350–355, 410 and 525 mu.

EXAMPLE 2

A culture medium for the production of coenzyme B_{12}, utilizing *Propionibacterium shermanii* as the vitamin B_{12}-activity producing microorganism, is prepared as follows: A sterile 20-liter Pyrex bottle is filled with 12 liters of distilled water at 30° C. To this are added 4 liters of sterile solution A, 700 ml. of sterile solution B, and 2 liters of sterile suspension C, the compositions of which are described hereinbelow, and sufficient sterile solution of sodium thioglycollate to give a concentrate in the culture medium of 0.05%. The bottle is rotated to mix the contents.

Sterile Solution A.—Preparation and Sterilization

Basamin (Anheuser Busch yeast extract)	g.	500
Glucose	g.	4800
$MgSO_4$, 2 M	ml.	80
$FeSO_4$, 0.2 M	ml.	32
$MnCl_2$, 0.1 M	ml.	16
Na_2MoO_4, 0.1 M	ml.	16
$CoCl_2$, 0.1 M	ml.	32
$CaCl_2$, 1 M	ml.	16

Distilled water to 32 liters.

To sterilize, place 4 liters of this solution in a 6-liter flask and sterilize for 45 minutes at 18 lb. steam pressure.

Sterile Solution B.—Preparation and Sterilization

KH_2PO_4 (reagent grade)	g.	170
$K_2HPO_4 \cdot 3H_2O$ (reagent grade)	g.	1,200

Distilled water to 5.6 liters.

To sterilize, place 700 ml. of this solution in a liter flask and sterilize for 45 minutes at 18 lb. steam pressure.

Sterile Suspension C.—Preparation and Sterilization

Powdered $CaCO_3$	g.	400

Distilled water to 2 liters.
Sterilize by heating 45 minutes at 18 lb. steam pressure.

This medium is inoculated with 750 ml. of an actively fermenting pure culture of said vitamin B_{12}-activity producing strain of *Propionibacterium shermanii* prepared by inoculating 5 mL of a culture of the bacteria into solution A supplemented with 0.05% cysteine·HCl and incubating this culture for 48–72 hours at 30° C. The 20-liter bottle is then filled with distilled water and incubated at 30° C. with stirring for a period of approximately 3–10 days. The pH of the medium is determined at frequent intervals and the pH is adjusted to pH 7, as required, by the addition of a solution of 2 N NaOH. In order to obtain more abundant growth, additional amounts of glucose are added at intervals as this compound is used up. At the end of the fermentation period, the cells are harvested by centrifugation and are obtained as a moist cell paste that can be used immediately for the preparation of coenzyme B_{12} or, if desired, can be frozen and stored for later use.

All operations are carried out in the dark or in very dim light.

Four kilograms of cell paste of *Propionibacterium shermanii* (obtained in accordance with the foregoing procedure are extracted with approximately 21 liters of 80–90% ethanol at boiling temperature. The filtered extract is concentrated in vacuo (45–50° C.) to remove alcohol. The resulting aqueous solution (volume 1800 ml.) is adjusted to pH 7 with 2 N NaOH passed through a 2 cm. diameter x 36 cm. high column of a sulfonic acid type cation-exchange resin (Dowex-50, 8x, 20–40 mesh) in the sodium ion form. The solution is then adjusted to pH 9.4 by addition of 2 N NaOH and is passed through a 2 cm. diameter x 30 cm. column of a quaternary ammonium type anion-exchange resin (Dow-

ex-2) in the hydroxide form. The combined effluent and washings (pH 9.8) are neutralized with glacial acetic acid to pH 6.2.

Phenol extraction.—The resulting solution (volume approximately 4 liters) is saturated with phenol and is extracted three times with 0.1 volume of 90% aqueous phenol, and the coenzyme is then displaced back into water by addition of 3 volumes of ethyl ether and 1 volume of acetone for each volume of phenol. The phenol-ether-acetone solution is extracted 3 times with 0.1 volume of water.

Chromatographic purification.—The resulting aqueous solution of coenzyme has a volume of approximately 600 ml. and a pH of about 7.0. The solution is acidified with 2 N HCl to pH 3.0. The solution is diluted with distilled water to 2 liters so that the final salt concentration is less than 0.02 M. The solution is then passed into a 2 cm. diameter x 80 cm. high column of a resin copolymer of styrene and divinyl benzene containing sulfonic acid groups in the mixed sodium ion-hydrogen ion form at pH 3 (Dowex-50K, 2x, 200–400 mesh; Dow Chemical Co.). The column is eluted successively at 3° C. with water (250 ml.) 0.03 M sodium acetate pH 5.5 (3,200 ml.) and 0.05 M sodium acetate pH 6.4 (3,600 ml.). The eluate is collected by means of an automatic fraction collector; each 10 minute fraction has a volume of approximately 25 ml. The coenzyme begins to elute after approximately 1 liter of pH 6.4 buffer has passed through the column. The elution of the coenzyme from the column is recognized by the appearance of a large absorbance peak at 260 mu, by the intense orange-red color of the eluate, and by the presence of coenzyme activity as indicated by the glutamate-mesaconate spectrophotometric coenzyme assay. The product is collected in approximately 40 fractions having a total volume of about 1 liter. From the 260 mu absorbance values and the estimated molar extinction coefficient of 35.5×10^6 cm.²/mole, the total quantity of coenzyme B_{12} compounds in the peak fractions is estimated to be approximately 500 μmoles. The coenzyme activities of the peak fractions are determined by the spectrophotometric coenzyme assay and the relative specific activities of the fractions calculated from these activities and the absorbance measurements at 260 mu on the respective fractions. The ultraviolet and visible absorption spectra of selected peak fractions are also determined. On the basis of the specific activity determinations and absorption spectra, the peak fractions with uniform properties are selected and combined.

The resulting coenzyme solution is extracted into phenol by the method described hereinabove and is displaced back into water by the addition of ether and acetone as also previously described. The aqueous solution thus obtained is extracted several times with ether to remove phenol and is then concentrated in vacuo. The final solution, containing approximately 400 μmoles of coenzyme B_{12} in a volume of about 40 ml., is deep red in color. The coenzyme B_{12} is crystallized by further concentrating the aqueous solution and allowing it to stand at 3° C. in the dark; or, alternatively, by adding to each volume of aqueous solution approximately 6 volumes of acetone and approximately 3 volumes of ether until the solution becomes slightly turbid and allowing the resulting mixture to stand at 3° C. until the coenzyme B_{12} crystallizes. The crystals are washed with 90% acetone, then with 100% acetone, and finally with ether, and the ether evaporated in vacuo to produce substantially pure crystalline coenzyme B_{12} in hydrated form.

The solution of coenzyme B_{12} in .03 M sodium acetate pH 6.7 shows absorption maxima at 260, 315, 335–337, 375 and 520–523 mu and the corresponding molar extension coefficients ($\times10^6$ cm.²/mole) of 35.5, 12.8, 12.5, 9.9 and 7.5, respectively. Analysis of the coenzyme B_{12} showed that it contains per mole of cobalt approximately one mole each of 5,6-dimethylbenzimidazole and adenine.

Treatment of the coenzyme B_{12} with 0.1 M KCN for approximately 30 minutes at room temperature results in virtually complete loss of coenzyme activity and results in the formation of a compound which in the region between 300 mu and 650 mu has an absorption spectrum substantially identical with that of the dicyano form of vitamin B_{12} with absorption peaks at 304 mu, 367 mu, 416 mu, 540 mu and 579 mu.

Exposure of the coenzyme B_{12} to visible light, such as a 100 w. tungsten filament lamp at a distance of 1 foot for a period of 30 minutes, results in virtually complete loss of coenzyme activity and results in the formation of a compound having an absorption spectrum very similar to that of hydroxocobalamin in the region between 320 mu and 600 mu and showing maxima at 350–355, 310 and 525 mu.

EXAMPLE 3

A culture of *Clostridium tetanomorphum* strain H1 is grown in accordance with the procedure described in Example 1 hereinabove (except that 5,6-dimethylbenzimidazole in a concentration of 1×10^{-4} M is used in place of the benzimidazole used in Example 1). The cells from 2 liters of fermented broth thus produced are harvested and treated in accordance with the phenol extraction and chromatographic purification method set forth in detail in Example 2 hereinabove, with suitable adjustment for the smaller quantities of starting material (approximately 8 g. cell paste—1/500 of the amount used in Example 2). The final aqueous solution obtained by combining the peak fractions from the elution of the coenzyme from the resin column, followed by extraction into phenol and displacement back into water, contains approximately 0.1 μmole of coenzyme B_{12}, as determined from the absorbance of its solution at 520 mu and the estimated molar extinction coefficient of 7.55×10^6 cm.²/mole.

The identity of the coenzyme B_{12} obtained in this example, with that obtained in Example 2 utilizing *Propionibacterium sharmanii*, is shown by the observation that they have the same absorption spectra; the same relative specific activity in the glutamate-mesaconate spectrophotometric coenzyme assay; undergo the same spectral changes and loss of coenzyme activity when exposed to cyanide ion or to light; and possess approximately 1 mole each of adenine, and 5,6-dimethylbenzimidazole per atom of cobalt.

Various changes may be made in carrying out the present invention without departing from the spirit and scope thereof. In so far as these changes and modifications are within the purview of the annexed claims, they are to be considered as part of my invention.

Having thus described my invention, what I claim and desire to secure by Letters Patent is:

1. A coenzyme B_{12} compound having a molecular weight within the range of about 1500 to 1700, a chemical structure like the cyano-benzimidazole-cobamides but lacking a cyano group and possessing an adenine moiety attached to the corphyrin portion of the molecule; characterized as being converted to the corresponding dicyano-benzimidazole-cobamide with removal of adenine and loss of coenzyme activity on treatmeat with cyanide ion and to the corresponding hydroxo-benzimidazole-cobamide-like compound with removal of adenine and loss of activity on exposure to light; characterized by the ultra violet and visible absorption spectrum of its solution in 0.03 M sodium acetate pH 6.7 as exhibiting a peak with the highest extinction coefficient at approximately 260 mu and lacking a prominent peak in the 350–367 mu region; and when in crystalline form being further characterized as forming prismatic crystals soluble in water, methanol, ethanol, and phenol, and substantially insoluble in acetone, ether and chloroform; said compound being further characterized as supporting the growth of the microorganism *Ochromonas malhamensis* and as having coenzyme activity as measured by the glutamatemesachonate spectrophotometric coenzyme assay.

2. The compound coenzyme B_{12}, an organic substance having vitamin B_{12} activity and coenzyme activity as measured by the glutamate-mesaconate spectrophotometric coenzyme assay; having a molecular weight within the range of about 1500 to 1700 and a chemical structure like that of vitamin B_{12} but lacking a cyano group and possessing an adenine moiety attached to the corphyrin portion of the molecule; characterized as being converted to dicyanocobalamin with removal of adenine and loss of coenzyme activity on treatment with cyanide ion and to a hydroxo-cobalamin-like compound with removal of adenine and loss of coenzyme activity on exposure to light; characterized by the ultra violet and visible absorption spectrum of its solution in 0.03 M sodium acetate pH 6.7 as exhibiting an inflection at 287 mμ and absorption maxima at 260 mu, 315 mu, 335–337 mu. 375 mu and 523 mu with corresponding molar extinction coefficients ($\times 10^6$ cm.^{2}/mole) of 35.5, 12.8, 12.5, 9.9 and 7.5 respectively, and as failing to exhibit a prominent absorption peak in the 350–367 mu region; and when in crystalline form being further characterized as forming prismatic crystals soluble in water, methanol, ethanol, and phenol, and substantially insoluble in acetone, ether and chloroform.

3. The compound benzimidazole-coenzyme B_{12}, an organic substance having vitamin B_{12} activity and coenzyme activity as measured by the glutamate-mesaconate spectrophotometric coenzyme assay; having a molecular weight within the range of about 1500 to 1700 and a chemical structure like that of the benzimidazole analog of vitamin B_{12} but lacking a cyano group and possessing an adenine moiety attached to the corphyrin portion of the molecule; characterized as being converted to dicyanobenzimidazole-cobamide with removal of adenine and loss of coenzyme activity on treatment with cyanide ion and to a hydroxobenzimidazolecobamide-like compound with removal of adenine and loss of coenzyme activity on exposure to light; characterized by the ultra violet and visible absorption spectrum of its solution in 0.03 M sodium acetate pH 6.7 as exhibiting an inflection at 280 mu and absorption maxima at 261 mu, 303–305 mu, 315 mu, 375 mu and 520 mu with corresponding molar extinction coefficients ($\times 10^6$ cm.^{2}/mole) of 35.5, 12.8, 12.8, 9.9 and 7.5 respectively, and as failing to exhibit a prominent absorption peak in the 350–367 mu region; and when in crystalline form being further characterized as forming prismatic dichroic crystals soluble in water, methanol, ethanol, and phenol, and substantially insoluble in acetone, ether and chloroform.

4. Coenzyme B_{12} as defined in claim 2 in substantially purified form.

5. Benzimidazole-coenzyme B_{12} as defined in claim 3 in substantially purified form.

6. Coenzyme B_{12} as defined in claim 2 in the form of its crystalline hydrate.

7. Benzimidazole-coenzyme B_{12} as defined in claim 3 in the form of its crystalline hydrate.

8. A process for the production of a vitamin B_{12}-active coenzyme B_{12} compound of the character set forth in claim 1 which comprises growing an *Ochromonas malhamensis* and B_{12} coenzyme-activity producing microorganism in an aqueous nutrient medium and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

9. A process for the production of coenzyme B_{12} of the character set forth in claim 2 which comprises growing an *Ochromonas malhamensis* and B_{12} coenzyme-activity producing microorganism in an aqueous nutrient medium and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

10. A process for the production of benzimidazole-coenzyme B_{12} of the character set forth in claim 2 which comprises growing an *Ochromonas malhamensis* and B_{12} coenzyme-activity producing microorganism in an aqueous nutrient medium containing benzimidazole and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

11. The process of claim 9 in which 5,6-dimethylbenzimidazole is incorporated in the nutrient medium.

12. A process for producing coenzyme B_{12} comprising: growing in an aqueous nutrient medium, Schizomycetes organisms of the genus Propionibacterium and capable of producing *Ochromonas malhamensis*-activity; and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

13. A process for producing coenzyme B_{12} comprising: growing in an aqueous nutrient medium, Schizomycetes organisms of the genus Streptomyces and capable of producing *Ochromonas malhamensis*-activity; and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

14. A process for producing coenzyme B_{12} comprising: growing in an aqueous nutrient medium, Schizomycetes organisms of the genus Pseudomonas and capable of producing *Ochromonas malhamensis*-activity; and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

15. A process for producing benzimidazole coenzyme B_{12} comprising: growing in an aqueous nutrient medium, containing benzimidazole, *Clostridium tetanomorphum* organisms capable of producing *Ochromonas malhamensis*-activity; and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

16. A process for producing coenzyme B_{12} comprising: growing in an aqueous nutrient medium containing 5,6-dimethylbenzimidazole, *Clostridium tetanomorphum* organisms capable of producing *Ochromonas malhamensis*-activity; and disrupting the resulting bacterial cells in the substantial absence of light and cyanide ion.

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EXPLORATIONS OF BACTERIAL METABOLISM

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H. A. Barker

Department of Biochemistry, University of California, Berkeley, California 94720

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EARLY YEARS

I grew up mostly in California, first in Oakland till the age of 11, and then in Palo Alto till I graduated from Stanford University. My parents had been part of the western migration. My father as a young man came to California from Maine, where he had grown up on a poor farm in a rural environment that was attractive to a child, but held little promise of a good life for an

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adult. He worked for a time as a farm hand in the San Joaquin Valley and later taught in an elementary school for a few years, until he could save a little money. In 1892 he entered Stanford with the first class, but had to drop out before graduating, for lack of funds. He returned to the public schools as a high school teacher. Later he became principal of a high school and eventually a public school administrator in several cities, including Oakland and Palo Alto, where I grew up.

My mother came to Stanford from Denver and obtained an A.B. degree in Classical Literature and an M.A. degree in Latin. She then taught languages in high school for a few years until she married. I had an older brother who was fond of literature and eventually became a professor of English. So there was nothing in my family background that predisposed me to a career in science with one possible exception. Both my father and mother were very fond of the outdoors and so each summer we spent a month or more, whenever possible, camping in the Sierras, and living a simple and quiet life in close contact with Nature. This resulted in my developing a considerable familiarity with plants and animals, and the physical environment, and perhaps even more important, developing a sense of satisfaction and accomplishment in relatively solitary activities such as fishing, hiking, and exploring new areas; this attitude was easily carried over to scientific work in a laboratory.

In high school I followed a rather standard college preparatory curriculum including mathematics, chemistry, and two foreign languages. The scholastic standards were not very high, so I had no difficulty getting adequate grades with little effort. One of my dominant interests in the last two years of high school was music. I had taken piano lessons for several years previously with only minimal results. My enthusiasm for music was greatly stimulated at this time by contacts and a developing friendship with a fellow student, Robert Vetlesen, who had unusual talents as a pianist, and at the age of 14 was already giving concerts of professional quality. As a result, I began to work hard to develop the techniques of piano playing, only to conclude after several years that my abilities in that direction are very limited. Although frustrating, this experience was beneficial in opening up to me the world of music from which I have derived much pleasure.

After graduating from high school I was fortunate to be able to spend a year (1924–1925) in Europe with my family. Most of the winter we stayed in Dresden, which at that time was a center of musical activity. I studied the piano, learned German, read classical German literature, and went to innumerable operas and concerts of every kind, usually occupying the cheapest seats. I remember that one of the highlights of the season was a musical festival honoring Richard Strauss on the occasion of his 60th birthday, during which he conducted several of his operas and ballets.

INTRODUCTION TO SCIENCE

I entered Stanford in 1925 with no idea which field I would ultimately choose as a major. Indeed, I inclined toward literary and historical subjects. Fortunately for me, much of the curriculum for the first two years was fixed, and I was required to take a course in general biology. I found much of the material both novel and interesting, and I recall that I was impressed by the enthusiasm and personalities of some of the instructors. So the following spring I decided to take a course in systematic botany from LeRoy Abrams. This turned out to be a good choice for me. The class was small and informal, and the work consisted mainly in collecting native plants in the adjacent fields and hills and learning to identify them by reference to Jepson's *Manual of Flowering Plants of California.* I soon began to appreciate the diversity of plants and the influence of environment on their distribution in nature. My knowledge in this area was later extended by taking a course in plant ecology and by accompanying a graduate student, Carl B. Wolf, on a seven-week field trip throughout the American southwest during which we collected over 5000 plants for the Stanford herbarium.

As a result of these experiences I decided, near the end of my sophomore year, that I would like to become some type of biologist. Since I had almost no background in the physical sciences, this decision meant that I had to start my real scientific education almost from the beginning. On examining the requirements for graduation in various fields, I found that I could obtain the physical sciences background I needed and fulfill the requirements for an A.B. degree most quickly by majoring in the School of Physical Sciences, which provided an introduction to mathematics, physics, chemistry, and geology. I studied all of these subjects with enthusiasm and graduated in the summer of 1929.

On entering graduate school I still had no definite idea as to which area of biology I should enter. So I decided to sample introductory courses in several areas, including plant and animal physiology, protozoology, and psychology. I found the protozoology course given by C. V. Taylor to be particularly stimulating because the class was very small and informal, which allowed close personal contact with an enthusiastic teacher. Also, because the emphasis was on microscopy, micro-manipulation, and other techniques I was able to learn something about the behavior and physiology of protozoa. The following spring I moved to the Hopkins Marine Station on the Monterey Peninsula, with a small group of premedical students, to study invertebrate zoology and embryology. The instruction was excellent and the environment was enchanting, but the most important thing that happened to me was a conversation with a fellow graduate student, Robert E. Hungate. He told me that he had been getting some instruction in

microbiology from a new member of the staff, a young Dutchman by the name of C. B. van Niel, whom he had found to be a superb teacher. On Hungate's advice I decided to ask van Niel to accept me as a student in the summer quarter. There was one complication. I had planned to start on a vacation in the Sierras somewhat before the end of the summer quarter. So I asked van Niel whether he could let me start the course a week early, in order to avoid interference with my vacation plans. He was rather surprised at this request, but the following day he agreed. I was van Niel's only student that summer; he spent much time with me introducing the experiments and discussing the results. On occasion the discussions would expand into lectures lasting an hour or two, which were presented with a clarity, enthusiasm, and almost hypnotic intensity that made a deep impression on me. I quickly became convinced that microbiology was a most exciting subject. One aspect of microbiology that van Niel emphasized was the developing knowledge and theories of the biochemistry of yeast and bacterial fermentations. Most of this material was quite new to me and I soon began to realize that my knowledge of chemistry was still insufficient to understand these phenomena. This realization was responsible, in considerable part, for my later decision to change my major to chemistry.

During this summer I also assisted Taylor with some experiments on the development of starfish eggs. Taylor was committed to spend the following academic year as a visiting professor of Zoology at the University of Chicago, and he invited me to come along as his research assistant. Since this provided an opportunity to see how I would like research in protozoology and to broaden my scientific background at another institution, I accepted.

At Chicago, Taylor suggested that I investigate some aspect of the conversion of active protozoa to their resting forms, or cysts, but left me free to decide which organism to use and how to proceed. After reading the available literature and making some trials, I decided to use the ciliate *Colpoda cucullus,* which can be cultured readily in an infusion of hay and forms cysts abundantly under appropriate conditions. Previous studies with various ciliates had suggested that several environmental factors, including food supply, pH of the medium, accumulation of excretion products, and lowered O_2 tension, may induce encystment; but there was little solid evidence to support any of these suggestions. Making use of some things I had learned from van Niel, I was able to simplify the conditions for encystment by culturing the ciliates on a suspension of bacteria in a nonnutritive medium and show that cyst formation is almost unaffected by pH or food supply, but that it is induced by other unidentified changes in the environment associated with crowding of the ciliates. These observations formed the subject of my first scientific publication. I also investigated the nature

of the factor in plant and animal infusions that induces the conversion of cysts to active ciliates, and was able to show that a number of common organic and inorganic compounds are inactive. Subsequently, Kenneth V. Thimann and I found that an acid ether extract of hay infusion contains much of the activity of the original extract. Thimann & Haagen-Smit (1) later established that the activity in ether extracts is attributable to the salts of l-malic and other organic acids.

While at the University of Chicago I also made some observations on the effect of moisture on the survival of *Colpoda* cysts exposed to high temperatures. Like bacterial spores, the cysts become more heat resistant when their moisture content is decreased. This led me to wonder whether the relation between moisture and heat resistance of living organisms could be attributed to an effect of moisture on the stability of cellular proteins. A search of the literature turned up only a few observations on this subject and so I decided this might be a suitable project to investigate later for a Ph.D. thesis. This could presumably be done in a chemistry department, where I could also increase my meager knowledge of chemistry in preparation for a career in microbiology. To do this I needed to find a sponsor who would accept me as a graduate student to work on this project. Fortunately, with the help of C. V. Taylor, I was able to persuade James W. McBain of the Stanford Chemistry Department to do so.

Before starting graduate work in Chemistry, I again spent the summer at the Hopkins Marine Station, this time as an assistant to J. P. Baumberger of the Stanford Department of Physiology. One of my duties was to serve as a teaching assistant in a small laboratory course in general physiology, and another was to investigate the toxicity of cyanide for the brine shrimp, *Artemia salina.* This remarkable creature had previously been observed to be relatively insensitive to cyanide, and I confirmed the fact that it can swim all day in a brine solution containing 50 mM KCN. However, the organism becomes sensitive to cyanide when the pH of the solution is lowered. By systematically varying the pH and the cyanide concentration we were able to conclude that the toxicity is determined primarily by the concentration of undissociated HCN. These observations were never published, but they were reported by Baumberger at a local scientific meeting.

That summer Leonor Michaelis spent several weeks at the Hopkins Marine Station as a visiting professor and I shared a small laboratory with him. Most of his time was spent either revising the manuscript of his book on oxidation-reduction potentials, or looking at the spectra of various dyes and natural pigments by means of what now seems like a rather primitive spectroscope. He also gave a few lectures on topics such as the theory and practice of electrophoresis. These were models of organization and clarity and greatly stimulated my interest in these areas of science. I bought his

books on mathematics, physical chemistry for students of medicine and biology, and hydrogen ion concentration and studied them with enthusiasm. At a later time I applied for a postdoctoral position with Michaelis at the Rockefeller Institute, but nothing was available.

I spent the following two years (1931–1933) working on my Ph.D. thesis, taking the required chemistry courses and examinations, and serving as a teaching assistant in a general biology course. For my thesis research I decided to use egg albumin, since Hopkins, Sørensen, and others had developed methods for the crystallization of this protein, and it had been used in some earlier experiments on heat denaturation. I soon learned that not all the useful information about the purification of egg albumin was to be found in the scientific literature. My first attempt to prepare the crystalline protein from six dozen eggs, obtained from a local store, was unsuccessful, apparently because the eggs were too old. The only useful product was some gold cake prepared from the yolks. A second preparation, starting with newly laid eggs, gave the expected crystalline product in good yield.

My plan to study the relation between water content and the heat stability of egg albumin worked rather well at first. By the application of simple methods, I was able to get satisfactory data on the effect of relative humidity on the rate of heat denaturation and also determine the water content of native and denatured egg albumin as a function of relative humidity. However, the interpretation of the kinetic data in terms of the chemistry of denaturation was not at all clear, and I could not think of any way of further elucidating the problem. McBain had given me much good advice about methods and other technical aspects of my research, but he lacked the background in protein chemistry to be helpful at this stage. I began to doubt that I had been wise to choose my own thesis problem and to wonder whether I had the ability to carry it through. But gradually I found my way out of this gloomy mood.

McBain had several good instruments for measuring physical properties, including a polarimeter and a mercury arc light source. Since the optical rotation of protein solutions had been reported to increase during denaturation, I decided to see how this property was affected by heating egg albumin solutions. The measurements were easy to make and the observed rotations were relatively large, but my early results were confusing because different egg albumin samples that initially showed the same specific rotation gave markedly different values after being heated. Only after a period of considerable frustration did I discover that by carefully standardizing the experimental conditions and having only a single variable I could get reproducible results. This was a valuable lesson that I never forgot. The explanation of my initial difficulties was that the specific rotation of the denatured protein was determined not only by the time and temperature of heating, but also by the initial pH and the protein concentration of the solution.

POSTDOCTORAL YEARS AT PACIFIC GROVE AND DELFT

I completed my Ph.D. thesis in the depths of the Great Depression and was fortunate to get a National Research Council Fellowship for a period of two years (1933–1935) to extend my biological training at the Hopkins Marine Station. Since I had previously acquired an interest in both marine organisms and microbiology I decided to attempt to isolate some marine diatoms and dinoflagellates and learn something of their physiology and metabolism. In the course of a year I was, in fact, able to isolate pure cultures of two species of diatoms and three species of small photosynthetic dinoflagellates, and to maintain several other species of dinoflagellates in species-pure culture. The diatoms were used for studies of photosynthetic quotients by means of Warburg's manometric techniques that I learned from van Niel and from Robert Emerson, who spent the summer at the Marine Station. The main conclusion derived from my experiments was that diatoms, like green algae, produce carbohydrates, rather than fats, as major products of photosynthesis. The dinoflagellate cultures were used mainly to determine environmental conditions favorable to the growth of these little-known organisms. The optimum temperature of the photosynthetic dinoflagellates was 18°C. This required them to be grown in a refrigerated bath that was illuminated by tungsten lamps that generated considerable heat. The result was an electricity bill for culturing these organisms that strained the very modest budget of the Marine Station. Partly for this reason, I terminated my studies of photosynthetic organisms and took up an investigation of the utilization of organic substances by a colorless alga, *Prototheca zopfii,* which van Niel had brought from Delft.

I found that *Prototheca* is an essentially aerobic organism that utilizes a large number of fatty acids and alcohols, and a few sugars, as substrates for respiration, but can also convert glucose to D-lactic acid in the absence of oxygen. While investigating the ability of cell suspensions to oxidize various substances, using manometric methods, I made the unexpected observation that the quantity of oxygen consumed was much smaller than that calculated for complete oxidation. With ethanol or acetate, for example, O_2 uptake was about 50% of theoretical, and with glycerol, the value was 29%. Carbon dioxide production was also very low, which indicated that a large fraction of each substrate was converted to other products. Since relatively little organic material accumulated in the medium, a major part of each substrate must have been assimilated in some form within the cells. An analysis of all the data I had collected on O_2 uptake and CO_2 production led to the conclusion that the assimilated material, corresponding to 50–80% of the substrates, had the empirical composition of a polysaccharide. This study directed the attention of microbiologists to the quantitative

importance of synthetic processes that are coupled with the aerobic degradation of organic substrates by cell suspensions of microorganisms. This so-called "oxidative assimilation," which is basically similar to the oxidative conversion of lactate to glycogen by muscle, was later shown by others to be a conspicuous feature of the aerobic metabolism of many bacteria and yeasts (2).

During my second year at the Hopkins Marine Station I had to consider what I would do when my fellowship ended. The job situation was very bleak. I applied for two positions in the Departments of Food Technology and Plant Nutrition at the University of California, but the prospects of their being funded for the following year were poor. At the same time, I answered an advertisement for a position as Junior Microbiologist in a US government laboratory, only to be informed that since I had taken only one course in microbiology I was not qualified. Fortunately, I also applied to the General Education Board of the Rockefeller Foundation for another fellowship to spend a year in the Delft Microbiology Laboratory with A. J. Kluyver, and in this I was successful. After spending a few weeks in the Sierra, my wife and I set off for Holland in July 1935.

Before leaving Pacific Grove I decided that I would like to investigate the anaerobic degradation of glutamate and the biological production of methane. These were both topics that I had learned about from van Niel. At that time a good deal was known about the bacteria responsible for several types of carbohydrate fermentation, but only a few observations, mostly with mixed cultures, had been made on the anaerobic degradation of amino acids by bacteria. van Niel thought that such studies should be done with pure cultures and that bacteria preferentially using particular amino acids could probably be obtained from soil or similar sources by the enrichment culture method. He had started anaerobic enrichment cultures using various single amino acids as energy sources and found that glutamate was a particularly good substrate. One of his students isolated a clostridium from a glutamate medium, but was unable to carry the work further; so I inherited the problem.

van Niel had done no experimental work on biological methane formation, but he had developed an ingenious hypothesis for the origin of methane, based mainly on the earlier experiments of Söhngen. The latter had shown that carbon dioxide is reduced to methane when molecular hydrogen is used as a second substrate and had also found that methane is the only hydrocarbon formed from a variety of organic substrates, irrespective of the number of carbon atoms they contain. van Niel concluded that in all these processes the organic substrate is oxidized to carbon dioxide and water, and this oxidation is coupled with the reduction of part of the carbon dioxide to methane. I decided to look for further evidence in support of this hypoth-

esis, and to attempt to isolate cultures of methane-forming bacteria, which had not been done previously.

When I first discussed these problems with Kluyver, he was sympathetic but, I think, a little skeptical that I could make much progress on either one during the year. He suggested that while getting started on these problems I should isolate a bacterium fermenting tartaric acid and possibly other C_4-dicarboxylic acids, and investigate the chemistry of the degradation of these compounds by the method developed in the Delft laboratory, namely, quantitative determination of the fermentation products. In fact, the isolation of a tartrate-fermenting strain of *Aerobacter aerogenes,* which could also ferment fumarate and l-malate, proved to be easy, and within a few months I had data on the fermentation products. The data were interpreted to mean that the dicarboxylic acids undergo an oxidation-reduction reaction to give succinate and an oxidized product, probably oxaloacetate, that is decarboxylated to pyruvate; the latter is presumably converted to various C_1 and C_2 products characteristic of *Aerobacter aerogenes* by reactions previously observed or postulated in other systems. No effort was made to detect the postulated intermediates or enzymes. This was considered not only too difficult, but also unnecessary for the purpose of establishing the pathway of the fermentation. Since the postulated pathway was consistent with the observed yields of fermentation products and since some of the component reactions had been demonstrated previously in other biological systems, we felt safe in assuming, without further evidence, that the postulated reactions occurred in these bacteria.

I made a similar study of anaerobic glutamate degradation by first isolating a clostridium, later identified as *Clostridium tetanomorphum,* that is capable of utilizing glutamate as a major energy source, and then determining the amounts of each product formed. I finally proposed a hypothetical sequence of reactions that might account for the observed products. The latter were ammonia, carbon dioxide, hydrogen, acetate, and butyrate, and the hypothetical pathway involved a more or less simultaneous deamination and decarboxylation of glutamate to form crotonate. Crotonate could presumably undergo reduction to butyrate and a coupled oxidation, by way of β-hydroxybutyrate and acetoacetate, to acetate and hydrogen. Again, no confirmatory evidence for the postulated pathway was obtained. As I later found, the pathway is incorrect in almost every detail for glutamate degradation by *C. tetanomorphum.* However, other investigators (3–5) have shown that *Peptococcus aerogenes* and other nonsporulating bacteria degrade glutamate by a pathway similar to that originally postulated for the clostridial fermentation. Although my study of glutamate fermentation did not contribute to knowledge of intermediary metabolism, it was useful in establishing the possibility of using single amino acids as energy sources for

anaerobic growth, and it eventually led to the discovery of an enzymatically active form of vitamin B_{12}.

While working on the degradation of C_4-dicarboxylic acids and glutamate, I also began to search for a way of testing van Niel's CO_2 reduction theory of methane formation with an organic substrate. Obviously what was needed was an organic compound that could be oxidized incompletely by methane-forming bacteria without producing carbon dioxide. The reduction of carbon dioxide to methane, if it occurred, could then be observed directly. A search of the literature turned up a short article by Omeliansky (6) which reported that a mineral medium containing ethanol and calcium carbonate, when inoculated with rabbit dung and incubated in the absence of O_2, undergoes a fermentation that produces gas containing mostly methane plus a little carbon dioxide. The high methane content of the gas suggested that ethanol was being oxidized only as far as acetic acid.

On the basis of this report, I started an enrichment culture for methane-producing bacteria under the conditions described by Omeliansky, but using an inoculum of sewage sludge, and soon obtained crude cultures that utilized ethanol rapidly according to the equation

$$2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COOH + CH_4.$$

The cultures were also shown to oxidize butanol to butyric acid, and the latter to acetic acid, both reactions being accompanied by a disappearance of carbon dioxide and the formation of an approximately equimolar quantity of methane. These results appeared to establish the validity of the CO_2 reduction theory of methane formation for these few substrates, and with the naiveté of youth I was immediately prepared to extend this concept to methane production from all other organic compounds. This was later found to be an oversimplification.

My observations on the methane fermentation of ethanol by enrichment cultures yielded another result that was destined to have a considerable influence on my career and the development of knowledge of fatty acid metabolism in later years. When handling various ethanol-methane enrichment cultures, I became aware that some had a slightly acidic odor, attributable to acetic acid, whereas others developed a much stronger, rancid odor. Steam distillation of volatile fatty acids from cultures of the latter type yielded substantial amounts of a relatively water-insoluble liquid organic acid that was identified as *n*-caproic acid. This was always accompanied by butyric acid. The formation of C_4 and C_6 fatty acids in high yields from ethanol in an anaerobic environment was an unexpected discovery that I reported to Kluyver with considerable excitement. Only after a careful search of the literature did I find that in 1868 a student of Pasteur, A. Bechamp, had observed the same phenomenon and reported the isolation of 75 g of caproic acid from a culture containing 106 g of ethanol (7)!

The publication of a report on this work was delayed for a year while an industrial company, to which Kluyver served as scientific adviser, investigated the possibilities of using the process for the commercial production of caproic acid. So far as I know nothing ever came of this. Nevertheless, the company provided me with a small retainer that made it possible, the following year, to start construction of a cabin in the mountains of California which we still use each summer.

A SOIL MICROBIOLOGIST AT BERKELEY

Toward the end of the year in Delft I accepted an appointment as Instructor in Soil Microbiology and Junior Microbiologist in the Division of Plant Nutrition of the Agricultural Experiment Station, University of California. As an instructor I at first assisted C. B. Lipman in teaching a laboratory and lecture course in soil microbiology that was required of all undergraduate students in the Soil Science curriculum, and later I was given sole responsibility for the course. Since my formal training in microbiology was slight, and my knowledge of soil microbiology in particular was even smaller, I had to work hard during the first years to learn enough about the subject to teach the fundamentals and those aspects that might be of some interest to students of soils. Fortunately, the students had reasonably good backgrounds in chemistry and general biology, although I found that because of the nature of the curriculum, they were generally more interested in the inorganic and physical properties of soils than in the microbial transformations of organic compounds. Since my interest was mainly in the latter area, a few years later I developed, in collaboration with Michael Doudoroff of the Bacteriology Department, and Reese H. Vaughn and Maynard A. Joslyn of the Food Technology Department, a new course in Microbial Metabolism in the Bacteriology Department that attempted to cover the knowledge of intermediary metabolism that was rapidly developing during that period. Later, Roger Y. Stanier and Edward A. Adelberg also participated in teaching this course, which attracted graduate students from several areas of biology.

Since I had an appointment in the Agricultural Experiment Station, I was supposed to make some contribution to agricultural research. The chairman of Plant Nutrition, Dennis R. Hoagland, asked me to join in the study of a nutritional disease of fruit trees and other plants, known as "little leaf." Shortly before my appointment, Hoagland and his associates had made the important discovery that this disease is caused by a deficiency of zinc, and he was actively engaged in investigating the conditions affecting the zinc requirement. Field observations seemed to indicate that little leaf symptoms were often particularly severe in areas, such as former corrals, that had received large amounts of animal manure; this suggested that microorgan-

isms are somehow involved in increasing the effect of zinc deficiency. Hoagland had begun to investigate this phenomenon by growing several successive crops of corn in pots of corral soil and had found that each successive crop grew more poorly, presumably because of increased zinc deficiency. Finally the condition became so severe that corn seeds would scarcely germinate. Hoagland asked me to see whether I could find any basis, microbiological or otherwise, for this phenomenon. I tried a number of experimental approaches, using sterilized and unsterilized soil, and soil reinoculated with various bacteria isolated from the original soil, but they led nowhere. Finally I made extracts of the soil to see whether they contained any material that would affect seed germination. It turned out that an extract was as poor a medium for germination as the original soil, and the explanation was that the salt concentration was just too high for corn. This terminated my experiments on corral soils. I did some other experiments on the effect of bacteria on the development and minor element nutrition of sterile plants grown in water culture but none of these produced any readily interpretable data. So with Hoagland's approval I abandoned research on bacteria-plant interrelations and devoted all my efforts to investigating simpler microbial systems.

The facilities available for microbiological research were very modest when I arrived in the Division of Plant Nutrition. They included an incubator room, a very old autoclave that did not always develop the expected temperature, a homemade oven for sterilizing glassware, a microscope, and a supply of test tubes and flasks. Most of the mechanical and electrical instruments that are now considered indispensible for research, such as centrifuges, colorimeters, respirometers, and pH meters, were lacking. Furthermore very little money was available in 1936 to purchase equipment of any sort. I well remember asking Hoagland whether I could order a $15 Seitz filter that I needed to sterilize media. He eventually approved my request but only after examining his budget to see whether we could afford it.

In part because of the limited facilities, my students and I initially concentrated on the isolation of various interesting kinds of anaerobic bacteria, which could be done with the available supplies. The bacteria included *Methanobacterium omelianskii,* the organism apparently responsible for the conversion of ethanol and carbon dioxide to acetate and methane; *Clostridium kluyveri,* responsible for the formation of butyric and caproic acids from ethanol; *Clostridium acidi-urici* and *Clostridium cylindrosporum,* which decompose uric acid and other purines; *Streptococcus allantoicus,* which degrades allantoin anaerobically; *Clostridium tetanomorphum* and *C. cochlearium,* which ferment glutamate; *Clostridium propionicum* and *Diplococcus glycinophilus,* which utilize alanine and glycine,

respectively; and *Butyribacterium rettgeri* and *Clostridium lactoacetophilum*, which ferment lactate in different ways. These organisms provided many of the biochemical problems I was to investigate in later years.

The isolation of each of the above-mentioned organisms involved some special problems, but none was as difficult as the initial isolation of *C. kluyveri*. I have already mentioned that some enrichment cultures for ethanol-utilizing, methane-forming bacteria produce considerable amounts of butyric and caproic acids. Microscopic examination of such cultures showed that they always contained a large spore-forming bacterium in addition to a smaller bacterium (*Methanobacterium omelianskii*) that was apparently responsible for the formation of methane. I undertook to isolate the spore-former by serial dilution in the same medium used for the enrichment cultures but with agar. It soon became apparent that isolated colonies of the spore-formers could not grow in this medium, since none was found beyond the second dilution, although *M. omelianskii* grew at much higher dilutions. As it seemed possible that the inability of the spore-former to grow in higher dilutions might result from the absence of suitable growth factors, I tried supplementing the medium with yeast autolysate and found that the addition of a very high level of this material would permit it to develop, though poorly. The problem then was to distinguish colonies of the caproic acid-forming clostridium from the many contaminating clostridia that thrived on yeast autolysate. This was eventually accomplished by using a remarkably sensitive but inexpensive instrument, my nose, to detect the presence of caproic acid in individual colonies picked with a micropipet. By these methods, I eventually isolated a pure culture of *C. kluyveri* but was disappointed to find that it produced little caproic acid in a yeast autolysate-ethanol medium. Considerable additional time and effort were required to find that the major essential nutrient derived from yeast autolysate is acetate and the minor nutrients are carbon dioxide, biotin, and *p*-aminobenzoate. When all these compounds were supplied, *C. kluyveri* grew readily, deriving energy from the conversion of ethanol and acetate to butyrate, caproate, and hydrogen (7).

EARLY EXPERIMENTS WITH RADIOACTIVE CARBON

I first became involved in experiments with radioactive carbon in 1939. Through my colleague Zev Hassid I met Sam Ruben of the Chemistry Department and Martin D. Kamen of the Radiation Laboratory, who had begun to use ^{11}C in the study of photosynthesis and dark CO_2 fixation by higher plants and algae. Ruben was the dynamic and tireless promoter of

^{11}C; and he was always interested in finding new biological systems to which the isotope could be effectively applied. When I pointed out that the carbon dioxide reduction theory of methane formation from organic compounds could be tested with $^{11}CO_2$, he was eager to collaborate.

Our experiments on the fermentation of ethanol by *M. omelianskii* confirmed the earlier conclusion that methane is derived from carbon dioxide and further demonstrated a considerable incorporation of carbon dioxide into cellular materials. An experiment on the fermentation of methanol by a *Methanosarcina* species was less convincing; although a small incorporation of carbon from carbon dioxide into both methane and cell material was observed, the results were not sufficiently quantitative to permit an unambiguous interpretation. This was a serious limitation of ^{11}C as a tracer. The 21-min half-life allowed only about 4 hr to prepare the $^{11}CO_2$, set up the experiment, carry out the incubation, separate the products, and prepare and count the final samples. The time was generally insufficient to get more than semiquantitative data. Despite this limitation we were later able to obtain useful data on the incorporation of carbon dioxide into acetate during the fermentation of purines by *C. acidi-urici* and into the carboxyl groups of propionic and succinic acids during fermentations by propionic acid bacteria.

The more complicated experiments with ^{11}C always involved a group effort. In order to reduce the duration of an experiment to a minimum it was necessary to plan every step of the preparative and analytical procedures ahead of time, and to make a dry run to be sure that everything necessary was available and working. Since our group had the lowest priority for use of the cyclotron, the actual experiments were always done at night and frequently could not be started before 1 AM. After the incubation, everyone was busy for a while carrying out some part of the separation procedure. Then we gathered about Ruben in the early hours of the morning to watch the counting of the samples. There was always a sense of excitement and drama when the incorporation of CO_2 into some metabolic product was shown by the high speed ticking of the counter. We felt that science was really progressing!

Carbon 14 was first prepared in significant amounts by Ruben and Kamen in 1940 (8), but because of wartime restrictions and the untimely death of Ruben, the isotope did not become available for experimental purposes until 1944. At that time T. H. Norris of the Chemistry Department and I recovered the ^{14}C from several hundred liters of saturated ammonium nitrate solution that had been exposed to stray neutron radiation from the 60-inch cyclotron. This was a messy job lasting several days. It involved boiling aliquots of the solution in a 12-liter flask, passing the vapors through a condenser and over hot copper oxide, and then absorbing the CO_2 in alkali

and precipitating it as $BaCO_3$. My share of the product was 1.8 g of $BaCO_3$ that had a rather low specific activity of about 1.5×10^5 cpm per mmole. This amount, small by current standards, proved to be sufficient for several fairly complicated tracer experiments on bacterial metabolism.

Although by this time I had some experience with tracer methodology, I knew virtually nothing about the technical aspects of estimating radioactivity because Ruben had previously always done the counting on a home-made counter that only he could operate. Fortunately for me, just about the time ^{14}C became available Kamen lost his position in the Radiation Laboratory because of wartime hysteria aroused by his conversation with a Russian consular official, and he was able to collaborate with me on the first tracer experiments with ^{14}C. He taught me the art of making mica window Geiger tubes and many other tricks of tracer methodology, and I in turn contributed something to his education in microbiology and biochemistry. It was a most useful and pleasant collaboration.

We first examined the role of carbon dioxide in the fermentation of glucose by *Clostridium thermoaceticum.* This bacterium had been shown to ferment glucose and xylose with the formation of over 2 moles of acetic acid per mole of sugar. The high yield of acetic acid, and the virtual absence of carbon dioxide or other one-carbon product, suggested that part of the acetic acid was formed from carbon dioxide. This hypothesis was shown to be correct by fermenting glucose in the presence of $^{14}CO_2$ and establishing that the isotope is incorporated into both carbon atoms of acetate, and that over 2 moles of carbon dioxide are actually formed and reutilized during the fermentation. Similar experiments showed that *Butyribacterium rettgeri* also uses carbon dioxide and converts it to acetic and butyric acids during the anaerobic degradation of lactate.

A somewhat more elaborate tracer experiment on the conversion of ethanol and acetate to butyrate and caproate by *C. kluyveri* provided substantial evidence that acetate, or a compound in isotopic equilibrium with acetate, is an intermediate in the conversion of ethanol to C_4 and C_6 fatty acids, and that caproic acid synthesis almost certainly involves the addition of a C_2 unit to the carboxyl carbon of butyrate rather than the reciprocal reaction (7). The latter conclusion was later confirmed by showing that ^{14}C-labeled caproic acid derived from [1-^{14}C]butyric acid and ethanol is labeled almost exclusively in the β-carbon atom.

SABBATICAL INTERLUDE

In 1941 I became eligible for my first sabbatical and was fortunate to receive a fellowship from the Guggenheim Foundation. I spent the first two months with L. F. Rettger at Yale University studying the fermentation products

and cultural characteristics of various nonsporulating anaerobic bacteria, which included an organism we later called *Butyribacterium rettgeri.* The last two months were spent with W. H. Peterson at the University of Wisconsin learning methods that had been developed there for investigating bacterial nutrition and assaying for growth factors by microbiological methods. The remainder of the year was spent with Fritz Lipmann in the Surgical Laboratories of the Massachusetts General Hospital. I had been attracted to Lipmann by his studies of enzymatic pyruvate oxidation by *Lactobacillus delbrueckii,* and by his stimulating review on phosphate bond energy. When I arrived he was engaged in the isolation of the labile phosphate compound formed from pyruvate that was soon shown to be acetyl phosphate. Lipmann determined the phosphate content of the isolated product and I contributed to its characterization by estimating the acetate content.

Before working with Lipmann all my research had involved the use of living bacteria, either as growing cultures or as cell suspensions. He introduced me to methods of preparing and studying cell-free extracts, and to techniques of detecting and estimating intermediate metabolites by colorimetric and other relatively sensitive procedures. The method that Lipmann favored for making bacterial extracts consisted of simply drying cells in a vacuum desiccator over P_2O_5 and then extracting them with buffer. This seems primitive by comparison with currently available methods, but it was inexpensive and served well for a number of later studies of bacterial enzymes at Berkeley.

SUCROSE PHOSPHORYLASE

On returning to Berkeley I continued to study various bacterial fermentations, some of which have already been mentioned, and also became involved in two new lines of research: a study of enzymatic sucrose degradation and an investigation of the deterioration of dried fruit during storage.

The investigation of sucrose degradation was initiated by Michael Doudoroff. He had isolated an H_2-oxidizing bacterium that also utilized a wide range of organic substrates. An interesting peculiarity of this organism, *Pseudomonas saccharophila,* was that it oxidized sucrose more rapidly than the component monosaccharides, glucose and fructose. About the time I returned from sabbatical leave Doudoroff came to the conclusion that further analysis of this phenomenon could only be made by the use of cell extracts. At my suggestion he made some dried cell preparations and soon

found that suspensions of the dried cells in a sucrose-phosphate solution caused a rapid esterification of inorganic phosphate. To identify and quantitate the products he enlisted the cooperation of Nathan O. Kaplan, who had had experience with the characterization of phosphate esters during his thesis research with David M. Greenberg, and W. Z. Hassid, who was a carbohydrate chemist. Together they demonstrated that the major enzymatic reaction is an apparently reversible conversion of sucrose and orthophosphate to fructose and glucose-1-phosphate. Because Hassid, Doudoroff, and I often had lunch together, and the conversation frequently dealt with the sucrose problem, I was gradually drawn into this research and contributed in various ways to the planning of the experiments and the isolation and characterization of sucrose and other disaccharides that can be synthesized by the phosphorylase from appropriate substrates (9). My most significant contribution to this research came as a result of an experiment that Doudoroff and I had planned to investigate the incorporation of ^{32}P into glucose-1-phosphate under various conditions. We incubated glucose-1-phosphate and $^{32}P_i$ with sucrose or fructose expecting that the reversible enzymatic reaction would result in the formation of labeled glucose-1-phosphate. Almost as an afterthought we included a control with only glucose-1-phosphate and $^{32}P_i$, and were surprised to find that more ^{32}P was incorporated into glucose-1-phosphate in the absence of the sugars than in their presence. In fact we did not believe the first result, and concluded that there had been a mix up of the samples. However, repetition confirmed the initial observation. We discussed the result for some time and by the next day reached the conclusion that the simplest interpretation was a reversible reaction of glucose-1-phosphate with enzyme to form a covalently bonded glucosyl enzyme and P_i. This soon led to the idea that sucrose was probably reacting in a similar way with the enzyme to form glucosyl enzyme and fructose. This in turn implied that the glucosyl moiety derived from sucrose could be transferred to another glucosyl acceptor such as sorbose to form glucosidosorboside in the complete absence of inorganic phosphate. Although I do not now recall the exact course of the discussion leading to these conclusions, I think that Doudoroff, who had a very agile mind, was the first to sense the probable explanation of our results. In any event, with Hassid's collaboration we were soon able to demonstrate the predicted synthesis of disaccharides by glucosyl transfer from sucrose in the absence of phosphate (10). These results established the concept that sucrose phosphorylase functions as a glucosyl-transferring enzyme, and provided substantial, though indirect, evidence for the existence of a covalent glucosyl enzyme compound, which was demonstrated many years later by Voet & Abeles (11).

RESEARCH ON DRIED FRUIT

Like many Americans in the early 1940s I felt an urge to assist in some way in the great conflict in which the nation was engaged. So in 1943 I eagerly accepted the invitation of my friend Emil M. Mrak of the Department of Food Technology to participate in a Quartermaster Corps project on methods of retarding the deterioration of dried fruit during storage, particularly since the work could be done on the campus and would not preclude other research activities. The project provided funds for an assistant; I was fortunate to select Earl R. Stadtman, a graduate of the Soil Science program who had taken my course in soil microbiology and later had assisted me in growing *Chlorella* on a large scale for Ruben. At first we knew almost nothing about the problems of preparing and storing dried fruit and soon discovered that the scientific literature dealing with these subjects was very meager. Mrak introduced us to the conventional methods of handling dried fruit, and then Stadtman and I, and later Victoria Haas, undertook a systematic study of factors influencing the deterioration of dried apricots. This required first the development of a reasonably quantitative measure of quality. Since fruit darkens progressively during storage this was accomplished by visually comparing the color of an alcoholic extract of fruit with a series of standards. We then proceeded to determine the effects of temperature, moisture, sulfur dioxide, and oxygen, and their interrelationships, on storage life, which was defined as the time required to reach an arbitrary degree of darkening (12). Several effects were revealed that had not previously been observed, or at least not adequately appreciated. Our results did not help to shorten the war, since they were not published until after its conclusion. I hope they have had some beneficial effect on the quality of commercial dried fruit, but I do not know that this is so.

CLOSTRIDIUM KLUYVERI: FATTY ACID METABOLISM AND AMINO ACID BIOSYNTHESIS

After the war Earl Stadtman decided to do his Ph.D. thesis with me and undertook to explore the enzymatic reactions participating in the energy metabolism of *C. kluyveri.* He soon found that crude extracts of dried cells are able to catalyze the anaerobic conversion of ethanol and acetate to butyrate and caproate, as well as the aerobic oxidation of ethanol and butyrate. This exciting discovery opened up the possibility of identifying the enzymatic reactions involved in the oxidation and synthesis of fatty acids. In fact the analysis of the system progressed rapidly. Stadtman found that acetyl phosphate is a product of the oxidation of both ethanol and butyrate in a phosphate buffer, and is an essential substrate for the synthesis of

butyrate when hydrogen is used as a reducing agent. Other significant findings were the discovery of an acetyl-transferring enzyme (phosphotransacetylase) and an enzymatic system for using acetyl phosphate to activate other fatty acids. Later, in Lipmann's laboratory, Stadtman and his associates showed that both of these enzyme systems require CoA and catalyze the formation of acyl-CoA compounds (13, 14).

Investigation of the utilization of several C_4 compounds that had been postulated to be intermediates in the reversible conversion of butyrate to acetate and acetyl phosphate established that acetoacetate can be either reduced to β-hydroxybutyrate or cleaved to acetyl phosphate and acetate, and that vinyl acetate can undergo a dismutation forming butyrate, acetyl phosphate, and acetate. However, tracer experiments showed conclusively that neither acetoacetate nor vinyl acetate could be an intermediate in butyrate oxidation or synthesis. Since no other C_4 compound at the oxidation levels of β-hydroxybutyrate and acetoacetate was used in this system, and no intermediate accumulated in sufficient amounts to be detected by the available methods, we were forced to the conclusion that the intermediates must be relatively stable complexes of C_4 compounds with a coenzyme or other carrier. This interpretation, which I first presented in a lecture before the Harvey Society in May 1950, was developed during discussions with Stadtman, and later with Eugene P. Kennedy, who spent a year with me as a postdoctoral fellow.

The following year I was invited to give a major lecture on the formation and utilization of active acetate at the first Symposium on Phosphorus Metabolism at Johns Hopkins University. I am not sure why I was selected for this assignment, although it was probably connected with the fact that Lipmann and Ochoa, who were major contributors to this area of research, were regarded at that time as competitors, and someone thought that selection of a neutral third party would be more diplomatic. In any event, I felt a great responsibility to present a comprehensive and balanced review of the whole field, covering the work that had been done with animal as well as bacterial systems. This required a major effort; I spent several months studying the literature and trying to arrive at a unified interpretation of the often incomplete and sometimes conflicting experimental results. Finally I reached the conclusion that acyl-CoA compounds are not only primary products of the oxidation of pyruvate and acetaldehyde, and primary substrates in the synthesis of acetoacetate and citrate, as had already been demonstrated, but that they must also be intermediates in the oxidation and synthesis of butyrate (15). I proposed a pathway for butyrate oxidation to acetyl phosphate via butyryl-CoA, vinylacetyl-CoA, β-hydroxybutyryl-CoA (by implication), acetoacetyl-CoA, and acetyl-CoA that was very similar to that later demonstrated experimentally by Lynen and others.

Vinylacetyl-CoA was postulated to be the initial oxidation product of butyryl-CoA because vinyl acetate is used more readily than crotonate by extracts of *C. kluyveri.* This apparently results from the specificity of the CoA transferase in this organism. Robert Bartsch later found that *C. kluyveri* contains a special isomerase that converts vinylacetyl-CoA to crotonyl-CoA.

Another aspect of the metabolism of *C. kluyveri* that proved to be of interest was the biosynthesis of its amino acids. Since *C. kluyveri* could be grown in a medium containing ethanol, acetate, and carbon dioxide as the only carbon compounds, other than small amounts of biotin and *p*-aminobenzoate, it was apparent that the cellular amino acids must all be synthesized from C_2 compounds and carbon dioxide. Tracer experiments by Neil Tomlinson showed that about 25% of the cellular carbon was derived from carbon dioxide and 75% from acetate. Examination of the 2-, 3-, and 4-carbon amino acids derived from the proteins of bacteria grown in the presence of $^{14}CO_2$ or [1-^{14}C] acetate established that the amino acid carboxyl groups are derived from carbon dioxide and the α-carbon atoms are derived from the carboxyl carbon of acetate. The results were consistent with the interpretation that *C. kluyveri* carboxylates acetyl-CoA and pyruvate to form pyruvate and oxaloacetate and then converts these compounds into the indicated amino acids. The postulated carboxylation reactions were subsequently demonstrated in *C. kluyveri* by Stern (16).

Tomlinson also investigated the origin of the carbon atoms of glutamate in *C. kluyveri* and found, in contrast to what had been observed in other organisms, that the α-carboxyl and β-carbon atoms are derived mainly from the carboxyl carbon of acetate, and that the γ-carboxyl carbon atoms are derived mainly from carbon dioxide (17). He pointed out that these results could be accounted for by the usual reactions for the conversion of oxaloacetate and acetyl-CoA to glutamate, provided the aconitase in *C. kluyveri* had an unconventional stereospecificity resulting in the formation of a double bond in *cis*-aconitate between the central carbon atom and the methylene carbon atom derived from oxaloacetate. This change in the position of the double bond would cause a reversal of the positions of the glutamate carbon atoms derived from oxaloacetate and acetate, as compared to glutamate formed by the usual tricarboxylic acid cycle reactions. This plausible hypothesis was eventually disproved by Gottschalk, who obtained convincing evidence that the citrate synthase, rather than the aconitase of *C. kluyveri,* displays an atypical stereospecificity. He found that *C. kluyveri* contains an (R)-citrate synthase rather than the (S)-citrate synthase characteristic of most other organisms. The (R)-citrate synthase of *C. kluyveri* fully accounts for the unusual origin of the carbon atoms of glutamate. This type of citrate synthase apparently occurs in only a few anaerobic bacteria (18).

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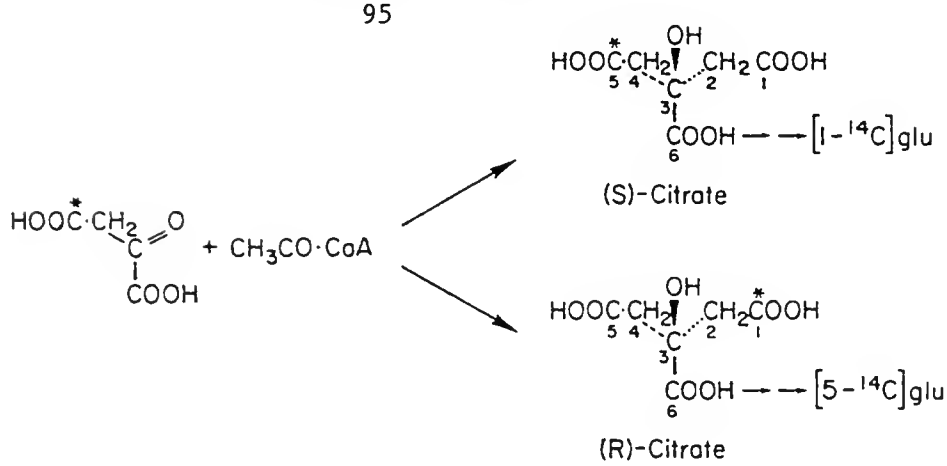


BIOCHEMISTRY OF METHANE FORMATION

Since our earlier tracer experiments with ^{11}C on the origin of methane in the fermentations of methanol and acetate had given equivocal results, when ^{14}C became available I decided to reinvestigate these problems. The immediate stimulus for this was a report by Buswell & Sollo (19) showing that little ^{14}C is incorporated into methane when unlabeled acetate is fermented in the presence of $^{14}CO_2$. This result was clearly contrary to the CO_2 reduction theory, but it did not specifically identify the source of methane carbon. I therefore encouraged Thressa Stadtman to study the fermentation of specifically labeled acetate; her results showed that virtually all the methane carbon is derived from the methyl group of acetate (20). She also established that methanogenic bacteria convert methyl alcohol to methane by a process not involving carbon dioxide reduction. In a further effort to define the chemistry of the conversion of acetate to methane Martin J. Pine investigated the fermentation of acetate labeled in the methyl group with deuterium, and found that the methyl group is incorporated as a unit into methane without loss of attached hydrogen or deuterium. The fourth hydrogen atom was shown to come from the solvent. These results appeared to exclude an oxidation-reduction of the methyl group during methane formation, although the possibility that the same hydrogen atoms are removed and returned to the methyl carbon cannot be entirely eliminated. Disregarding this possibility, the results of the various tracer experiments are consistent with a simple decarboxylation of acetate to methane and carbon dioxide. However, this still seems unlikely since it is difficult to imagine how an organism can obtain useful energy from such a process. As yet no one has succeeded in obtaining a cell-free extract with which to make a further analysis of the chemistry of the conversion of acetate to methane.

In 1956 I undertook to summarize the results of our studies on methane fermentation and to correlate this with the contributions of other groups.

This led to the proposal of a generalized pathway for the formation of methane from either acetate, methanol, or carbon dioxide, all of which are known to be used by some methane-forming bacteria (20). The main features of this pathway were the carboxylation of an unspecified carrier and the sequential reduction of the carboxyl group to a methyl group that was finally converted to methane. The methyl groups of acetate and methanol were postulated to enter this sequence by a more or less direct methyl transfer to the carrier and be either reduced to methane or oxidized to carbon dioxide by a reversal of the carbon dioxide reduction pathway, or both. This conceptual scheme seems to have been of some value to later students of methane fermentation (21).

TRANSITION FROM MICROBIOLOGY TO BIOCHEMISTRY

Since my original position at Berkeley was that of a soil microbiologist and I ended up as a biochemist, I should mention some of the stages of my metamorphosis. I remained a member of Plant Nutrition until 1950. At that time, following the death of D. R. Hoagland, its long-time chairman, five members of the faculty—Zev Hassid, Paul K. Stumpf, Eric E. Conn, Constant C. Delwiche, and I—whose interests were primarily biochemical, formed a new Department of Agricultural Biochemistry in the College of Agriculture. When the Biochemistry and Virus Laboratory was completed in 1951 we moved in along with the new Biochemistry Department and the Virus Laboratory. Although the laboratories were an improvement over those we had previously occupied, the administrative arrangements in the building were difficult for several years because of an almost constant struggle over authority and space. This situation was greatly ameliorated when Esmond Snell became chairman of the Biochemistry Department. Shortly thereafter Hassid and I transferred into that department, and the other members of Agricultural Biochemistry moved to the Davis campus of the University to establish a new, and now flourishing, Department of Biochemistry and Biophysics. In 1964 the remaining interdepartmental problems were resolved by moving the Biochemistry Department to a new building.

From 1936 to 1948 my students obtained advanced degrees in the graduate curricula of Bacteriology, Microbiology, or Agricultural Chemistry. The Biochemistry Department at Berkeley during that period was part of the Medical School; graduate degrees in biochemistry were not available to students studying with other faculty members. Since many students in other departments were doing research on biochemical problems and wished to be recognized as biochemists, there was considerable interest among both

students and faculty in setting up an academic mechanism for giving degrees in biochemistry outside of the Biochemistry Department. I. L. Chaikoff of the Physiology Department and I took the lead in organizing an interdepartmental group major, called Comparative Biochemistry, to take care of this problem. A curriculum for a Ph.D. degree in Comparative Biochemistry was approved in 1948 and from then until 1958, when I joined the new Biochemistry Department, most of my students majored in this field. I took on the responsibilities of graduate student adviser in Comparative Biochemistry when the group was organized, and retained the position until my academic retirement in 1975. During this period about 75 students obtained Ph.D. degrees in Comparative Biochemistry. Subsequently many of these students have contributed substantially to the world of biochemistry; notable examples of graduates from the earlier years of this program are Elizabeth F. Neufeld, Paul A. Srere, and Earl Stadtman.

THE BR FACTOR

I have previously mentioned some experiments on *Butyribacterium rettgeri*, an anaerobic bacterium that catalyzes butyric acid fermentation of lactate and carbohydrates. In 1950 one of my students, Leo Kline, tried to grow the organism in a synthetic medium and found that it required a small amount of yeast extract in addition to the then known nutrients and growth factors. An examination of some properties of the essential material, called the BR factor, established that it was a very stable carboxylic acid, readily extractable with organic solvents from acid aqueous solutions; in addition, it occurred in several more complex forms that were not soluble in organic solvents until released by vigorous acid or alkali hydrolysis. These properties were similar to but not identical with those of some other unidentified growth factors, including a *Lactobacillus casei* factor studied by Guirard, Snell & Williams (22), and a pyruvate oxidation factor for *Streptococcus faecalis* reported by O'Kane & Gunsalus (23). At this stage, I should have contacted these investigators in order to make a closer comparison of the various preparations. Instead, after Kline had completed his thesis, I continued work in the isolation of the BR factor. I obtained about 100 pounds of *Penicillium notatum* mycelium, a good source of BR factor, prepared many gallons of autolyzate, acid hydrolyzed the material in an autoclave, built a large liquid-liquid extractor, extracted the hydrolyzate for weeks, and with the aid of an assistant, performed innumerable tedious and not always completely reproducible assays. After some additional steps, we obtained several hundred milligrams of material substantially purified but still containing a number of components both active and inactive. About this time Gunsalus visited Berkeley and in the course of conversation we

found that the properties of the BR factor and the pyruvate oxidation factor were very similar. By exchanging samples we found that they were in fact identical. Since Gunsalus' preparations were considerably purer than ours, I immediately abandoned the attempt to further purify the BR factor. Subsequent observations demonstrated that lipoic acid is highly active as a growth factor for *B. rettgeri.*

The lipoate requirement of *B. rettgeri* continued to be of interest because the function of the factor appeared to be different from that in other organisms. Lipoate had been shown to function as an electron carrier in the oxidation of pyruvate. Kline and others found on the contrary that *B. rettgeri* does not require lipoate for the utilization of pyruvate, but only for the utilization of lactate. Since the products formed from lactate and pyruvate are qualitatively the same, it was concluded that lipoate probably functions as an electron carrier in the oxidation of lactate to pyruvate. Martin Flavin became interested in the role of lipoic acid in this system when he was in my laboratory, and later collaborated with C. L. Wittenberger in a study of this problem. They reached the tentative conclusion that in lactate oxidation, enzyme-bound lipoate mediates electron transfer between an unidentified electron carrier and DPN (24). Further analysis of the specific role of the lipoate-containing enzyme in the lactate-oxidizing system in *B. rettgeri* has been impeded so far by the instability of the system (25).

PURINE DEGRADATION BY CLOSTRIDIA

From 1937 to 1957 one of my major research interests was the degradation of uric acid and other purines by clostridia. I started on this project as a result of a conversation with a colleague who raised chickens. He had filled a large container with chicken droppings, which contain uric acid, and water, and was greatly impressed by the rapid rate at which the mixture developed a strong ammoniacal odor. I undertook the isolation of the responsible bacteria and had no difficulty in obtaining a number of cultures that showed a high degree of specificity for the degradation of uric acid and a few other purines. Jay V. Beck, my first graduate student, joined me in studying the physiology and nutrition of the bacteria, which we named *Clostridium acidi-urici* and *C. cylindrosporum,* and in identifying the fermentation products. We found that both organisms decompose uric acid, xanthine, and guanine readily, and hypoxanthine more slowly, with formation of acetate, carbon dioxide, and ammonia as major products; in addition, *C. cylindrosporum* forms significant amounts of glycine. Later, Norman Radin found that formate is also a fermentation product. Since both clostridium species were able to activate glycine as a reducing agent and decom-

pose it when uric acid was simultaneously available, glycine appeared to be a normal intermediate in purine degradation. Various enzymes and metabolites known to participate in the aerobic degradation of purines were not detected in the clostridia, and consequently we concluded that the pathway of purine degradation in these bacteria is quite different from that in aerobic organisms. This conclusion was strengthened by a number of tracer experiments on the origin of the product carbon atoms. The early experiments showed that both carbon atoms of acetate and the carboxyl group of glycine are derived in part from carbon dioxide. Later experiments by Jon L. Karlsson and by Jesse C. Rabinowitz with specifically labeled purines, glycine, and formate established a similarity between the pathways of purine degradation by clostridia and of purine biosynthesis by other organisms (26). The pieces of the jigsaw puzzle of the degradative pathway were finally assembled into a coherent picture as a result of enzymatic studies initiated by Radin and carried to completion by Rabinowitz. Radin found that the first step in uric acid utilization is its reduction to xanthine, which is then converted by crude enzyme preparations to glycine, formate, carbon dioxide, and ammonia. Glycine can be oxidized to acetate, carbon dioxide, and ammonia, and serine is converted by way of pyruvate to the same products. These results, in conjunction with those of the tracer experiments, suggested that acetate is formed mainly by the sequence glycine → serine → pyruvate → acetate. Radin also obtained presumptive evidence for the formation of one or more aminoimidazoles, none of which was identical with 4-amino-5-carboxamidoimidazole, which had been implicated in purine biosynthesis. These observations suggested that the pyrimidine ring of xanthine is initially cleaved at the 1–6 bond to yield 4-ureido-5-carboxyimidazole. The formation of this intermediate was confirmed by Rabinowitz, who then proceeded to elucidate the further enzymatic steps in purine degradation, including the role of folic acid, in elegant detail (26, 27). The last contribution to this area of research from my laboratory was a study by Willard H. Bradshaw of the properties, particularly the substrate specificity, of the xanthine dehydrogenase of *C. cylindrosporum,* the enzyme responsible for the reduction of uric acid to xanthine.

SABBATICAL AT THE NATIONAL INSTITUTES OF HEALTH

As a result of my early experience with ^{14}C, I had come to rely heavily on the application of tracer methods to intact cells for the elucidation of various problems of bacterial metabolism. When the use of intact cells seemed inadequate, I occasionally encouraged my students to use cell-free extracts, but until the early 1950s we did not attempt to purify specific

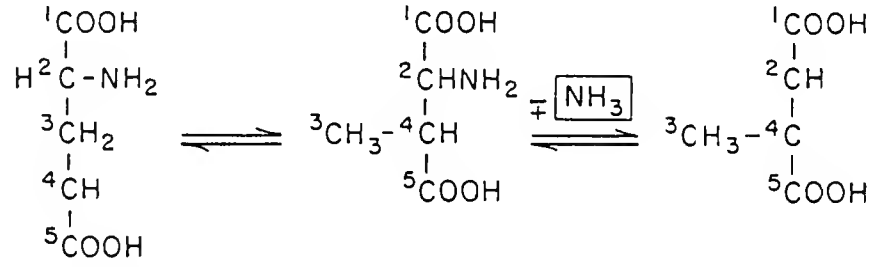
enzymes. The stimulus to investigate individual reactions of metabolic pathways through the use of purified enzymes was provided by Arthur Kornberg, who spent part of the summer of 1951 in my laboratory learning how to handle anaerobic bacteria. He spoke with such enthusiasm about the advantages of using purified enzymes that I decided I should get some experience in the art of enzyme isolation. The following year I spent six months with Kornberg at the National Institutes of Health. He and I shared a small laboratory and I was able to draw upon his knowledge and experience whenever it was required. I learned a great deal from him in a few months that I was later able to apply to my own research.

In Kornberg's laboratory I investigated two unrelated problems. One was the purification of the coenzyme A transferase from *C. kluyveri,* using an assay method developed by Earl Stadtman. I tried every known method of enzyme purification on this transferase, but even with Kornberg's advice I had very little success; the best preparation was purified only about fivefold with a 31% yield. However, even the methods that did not give any purification provided valuable experience, and that was what I needed. My second research problem, suggested by Kornberg, was the isolation and characterization of ATP from the sulfur-oxidizing bacterium, *Thiobacillus thiooxidans,* which had been reported to differ from the ATP of other organisms by having the phosphate groups attached to the 3', rather than the 5' position of adenosine. During the isolation of ATP I learned how useful ion exchange resins can be for separating charged molecules, and during the characterization of ATP I came to appreciate the value of enzymes as specific and convenient analytical reagents. The conclusion of our work was that the ATP of thiobacillus is the same as that of other organisms.

GLUTAMATE FERMENTATION AND B_{12} COENZYMES

I have already mentioned my early studies on glutamate degradation by *Clostridium tetanomorphum.* Further investigation of the chemistry of this process was put off for many years while I was involved in what seemed to be more exciting problems. A stimulus to return to a study of glutamate metabolism was provided indirectly by Kornberg, who isolated a histidine-degrading strain of *C. tetanomorphum* while visiting my laboratory. My student, Joseph Wachsman, investigated the early steps of histidine degradation by this organism and concluded that glutamate is an intermediate in this process, as it is in histidine degradation by aerobic organisms. He then studied the degradation of glutamate by both tracer and enzymatic methods and showed that the carbon chain is cleaved between carbon atoms 2 and 3 to form acetate from carbon atoms 1 and 2, and pyruvate from

carbon atoms 5, 4, and 3 (26). The pyruvate is oxidized to carbon dioxide (carbon 5), hydrogen, and presumably acetyl-CoA (carbon atoms 4 and 3), which is mainly converted to butyrate. These results established that glutamate was being degraded by a novel pathway. A clue to the nature of the pathway was provided when Wachsman identified mesaconic acid, a branched-chain unsaturated dicarboxylic acid, as an intermediate in glutamate degradation.



The nature of the carbon skeleton rearrangement in the glutamate-mesaconate conversion was established a little later by Agnete Munch-Petersen, who converted [4-^{14}Cl] glutamate enzymatically to mesaconate and determined the position of the isotope in the product. The result proved that the bond between carbon atoms 2 and 3 of glutamate is broken, and a new bond is established between carbon atoms 2 and 4, leaving carbon atom 3 in a methyl group. A further study of the carbon chain rearrangement established that the first product formed from glutamate is the amino acid 3-methyl-L-aspartate, which is then deaminated to form mesaconate. The inter-conversion of glutamate and 3-methylaspartate proved to be the most novel and interesting step in glutamate degradation. The branched-chain amino acid was missed in the early investigations of this system because some of its properties are very similar to those of glutamate, and because the equilibria in the system are unfavorable for its accumulation in quantity. It was first detected as a product of mesaconate amination only after we found that the enzyme catalyzing its reversible conversion to glutamate can be inactivated by treatment with charcoal.

A rather detailed account of the circumstances leading to the isolation of the charcoal-absorbable cofactor for the mutase and its identification as a derivative of vitamin B_{12} has recently been published (28) and need not be repeated here. But perhaps a few comments may be of interest. In retrospect, the isolation of the corrinoid coenzymes was rather straightforward once we had reached an adequate level of understanding of the enzymatic system in which it functioned. We had a specific, sensitive, and

reasonably convenient enzymatic assay; the coenzyme was relatively stable except to one environmental factor, and its physical properties were ideally suited to permit purification by ion exchange and solvent extraction techniques. Nature had put only one roadblock in our way, namely, the instability of the coenzyme to light. Our failure to recognize this property caused much difficulty and frustration during the early stages of our investigation, which lasted almost two years. Once this property was recognized, the isolation of the coenzyme could be completed in a few weeks. The critical factor for the recognition of the light effect was the development of a rapid spectrophotometric assay for the coenzyme. We should have done this much earlier, but the advantages of such an assay were not as evident at the time as they are in hindsight.

I cannot leave this topic without at least mentioning my associates, students and postdoctoral fellows, who made important contributions to the successful outcome of our work on the isolation and characterization of corrinoid coenzymes. Agnete Munch-Petersen first undertook to purify the coenzyme and established some of its ionic properties; Herbert Weissbach first recognized the coenzyme to be a corrinoid compound and contributed in many ways to the identification of its structure; Harry Hogenkamp determined the structure of the two nucleotides formed by photolysis of the coenzyme; John Toohey established optimal conditions for corrinoid coenzyme formation by *C. tetanomorphum,* and isolated and characterized several coenzyme analogs from bacteria and liver; Benjamin Volcani developed a bioautographic method for the identification of small amounts of coenzyme analogs; Jeff Ladd determined the pK_a values of the coenzymes and the effect of ionization on the absorption spectra; David Perlman of the Squibb Institute for Medical Research assisted us greatly by providing large quantities of propionic acid bacteria containing various coenzyme analogs; Axel Lezius identified the major corrinoid coenzyme in a methane-producing bacterium; Roscoe Brady demonstrated the reactions involved in the adenosylation of corrinoid compounds in *Propionibacterium shermanii;* and Robert Smyth assisted in many ways with the assay and initial isolation of the coenzymes.

While studies of the structure of the corrinoid coenzymes were progressing, we simultaneously tried to learn something about the chemistry of the mutase reaction, but with little success. Attempts to detect either free or coenzyme-bound intermediates gave negative results, so we concluded that they must have a very short life. A somewhat more significant conclusion was reached in an investigation of hydrogen transfer during the mutase reaction. Arthur Iodice found that solvent hydrogen is not incorporated into products in appreciable amounts; this supported the interpretation that hydrogen is transferred as either H^0 or H^-, but not as H^+. After Lenhert & Hodgkin (29) showed the presence of a deoxyadenosyl group in the

coenzyme, Fujio Suzuki and I investigated the role of the coenzyme as a hydrogen-transferring agent by looking for a transfer of tritium from [^{3}H-methyl]3-methylaspartate to coenzyme. A significant amount of tritium was found in the coenzyme; unfortunately, the coenzyme from a control experiment without enzyme showed about half as much tritium, so the results were ambiguous. Not long thereafter Abeles and his associates clearly demonstrated that the coenzyme functions as a hydrogen-transferring agent by the use of synthetic, tritium-labeled coenzyme in the diol dehydrase reaction. We later confirmed that the coenzyme functions in the same way in the glutamate mutase reaction.

To learn more about the mode of action of glutamate mutase I felt it would be desirable to have a highly purified preparation. This turned out to be more complicated than I anticipated. Early attempts to purify the activity showed that it depends on the presence of two readily separable proteins which we called the E and S components. The relatively unstable E component, with a molecular weight of about 125,000, was purified by Suzuki; and the relatively stable S component, with a molecular weight of 17,000, was purified by Robert L. Switzer. Although we learned something about the molecular and kinetic properties of these proteins and the conditions for their interaction, we were unable to demonstrate separate functions for the two subunits, if such they be, or understand how they interact to form the catalytically active species. This remains a problem for the future.

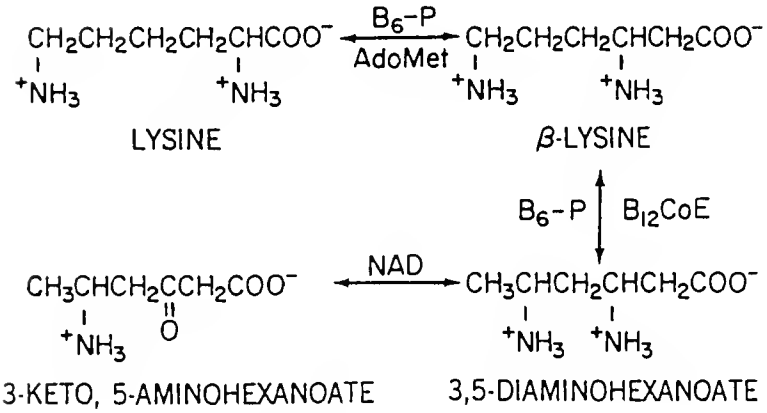
After the discovery of the role of corrinoid coenzymes in the glutamate mutase reaction, I considered the possibility that they might also participate in the methylmalonyl mutase reaction, but never got beyond the stage of speculation. Soon afterward, several groups of investigators demonstrated that the coenzyme is indeed required for this reaction. Another process in which vitamin B_{12} had been implicated by the nutritional experiments of Snell, Kitay & MacNutt (30) was the conversion of ribonucleotides to deoxyribonucleotides in *Lactobacillus leichmannii.* When Raymond Blakley came to my laboratory I encouraged him to see whether corrinoid coenzymes participate in this conversion. He was able to obtain a cell-free preparation that reduced the ribose moiety of CMP to a deoxyribose moiety and established that the reaction is strongly stimulated by corrinoid coenzymes. After returning to Canberra, Blakley purified the ribonucleotide triphosphate reductase responsible for deoxyribose formation and clarified the novel role of the coenzyme in this oxidation-reduction reaction.

LYSINE DEGRADATION BY CLOSTRIDIA AND RELATED PROBLEMS

In 1962, Olga Rochovansky came to my laboratory as a postdoctoral fellow and said she would like to investigate the anaerobic degradation of lysine

while getting experience in handling anaerobic bacteria. The year before, Thressa Stadtman (31) had reported that cell-free extracts of *Clostridium sticklandii* are able to convert lysine to acetate, butyrate, and ammonia. She had identified several cofactors required for the reaction, but had been unable to detect any intermediate in lysine degradation, even when one or another of the cofactors was omitted from a reaction solution. Since it seemed possible that another organism might provide enzyme preparations more suitable for detecting intermediates, after consultation with Stadtman, Rochovansky undertook to isolate a lysine-degrading anaerobe. She succeeded in obtaining such an organism (*Clostridium* SB4) from sewage sludge and went on to show that the cofactor requirements for lysine degradation by extracts are almost the same as for *C. sticklandii*.

The search for intermediates in lysine degradation by extracts of SB4 was started by my student, Ernest A. Rimerman. We decided to begin by adding all the known cofactors except coenzyme A in the expectation that intermediates found before the CoA-dependent reaction might accumulate in larger amounts than those formed subsequently. Rimerman soon found that omission of CoA caused the accumulation of significant amounts of a heat-labile neutral compound that could be separated from other products by paper electrophoresis. This compound was identified as 3-keto-5-aminohexanoic acid, an unexpected product to be derived from lysine, which is substituted in the 2 and 6 positions. An explanation for the location of the carbonyl group was obtained by Ralph N. Costilow who was visiting my laboratory. He looked for other intermediates in lysine degradation by omitting DPN from an otherwise complete reaction solution, and by paper ionophoresis at neutral pH he detected a second basic amino acid that overlapped lysine. At a lower pH this amino acid separates readily from lysine and can be easily assayed. The new amino acid was isolated and identified as L-3,6-diaminohexanoic acid (β-lysine), a compound previously known only as a component of some polypeptide antibiotics. The formation of this amino acid indicated that the first step in lysine degradation is a migration of the amino group from the 2 to the 3 position. This was later established more firmly after purification of the responsible enzyme, L-lysine aminomutase, by Thomas P. Chirpich; he also demonstrated that the enzyme is stimulated by pyridoxal phosphate, ferrous ion, and S-adenosylmethionine. The second intermediate in anaerobic lysine degradation, and the immediate precursor of 3-keto-5-amino hexanoate, was soon found to be 3,5-diaminohexanoate. This compound was first recognized by Stadtman & Renz (32); it was independently discovered in my laboratory by Eugene E. Dekker while looking for an intermediate accumulating in the absence of corrinoid coenzyme. Thressa Stadtman and her associates at the National Institutes of Health later purified and extensively investigated the corrinoid coenzyme-



dependent enzyme responsible for the formation of 3,5-diaminohexanoate, whereas we concentrated on the enzymes responsible for the formation and degradation of 3-keto-5-aminohexanoate.

The enzyme catalyzing the oxidative deamination of 3,5-diaminohexanoate to the 3-keto acid was purified by John J. Baker and shown to be a highly substrate-specific, but otherwise conventional, dehydrogenase. Su-Chen L. Hong and Ing-Ming Jeng found that the degradation of 3-keto-5-aminohexanoate requires the presence of acetyl-CoA, but the nature of the enzymatic reaction responsible for the degradation eluded us for some time. The acetyl-CoA requirements suggested that the degradation would follow the usual pathway for fatty acid oxidation; formation of a CoA thioester of the β-keto acid followed by a thiolytic cleavage, which in the lysine degradation system would result in the formation of 3-aminobutyryl-CoA and acetyl-CoA. However, numerous attempts to detect the postulated intermediates and products were unsuccessful.

At this point we decided to switch to another experimental approach to the problem, namely, the synthesis of the postulated 3-aminobutyryl-CoA and the test of its ability to be further degraded. Jeng soon found that extracts of our lysine-fermenting clostridium contain a highly active deaminase that converts L-3-aminobutyryl-CoA to crotonyl-CoA. The presence of this enzyme and crotonase accounted for our earlier inability to detect 3-aminobutyryl-CoA as a product of 3-keto-5-aminohexanoate degradation, but did not account for our failure to detect the other possible intermediate, 3-keto-5-aminohexanoyl-CoA. The nature of the reaction responsible for the removal of 3-keto-5-aminohexanoate in the presence of acetyl-CoA was finally determined by Takamitsu Yorifuji, who purified the responsible 3-keto-5-aminohexanoate cleavage enzyme, and found to our surprise that it catalyzes the following reaction:

$$CH_3\underset{\underset{NH_3^+}{|}}{C}HCH_2COCH_2CO_2^- + Ac\cdot CoA \rightleftharpoons CH_3\underset{\underset{NH_3^+}{|}}{C}HCH_2CO\cdot CoA + AcAcO^-.$$

This is a previously unrecognized type of reaction for the degradation and synthesis of β-keto acids.

Since the study of lysine degradation by clostridia had turned up several novel types of reactions, I decided to investigate analogous enzymatic reactions in two aerobic bacteria that utilize β-lysine or 3,5-diaminohexanoic acid as an energy source. Although these investigations are not yet complete, studies by Henry N. Edmunds, Su-Chen L. Hong, Gerhard Bozler, John M. Robertson, and Masahiro Ohsugi have established that the type of β-keto acid cleavage reaction discovered in clostridia also occurs in both aerobic bacteria. The β-lysine decomposing organism is of additional interest because it catalyzes both an initial acetylation of the substrate and a novel but as yet not fully defined type of deacetylation reaction at a later step in the degradation sequence.

FINAL COMMENTS

It will be obvious to the reader that the central focus of my scientific career has been the exploration of bacterial metabolism, generally the energy metabolism of anaerobic bacteria, with the objective of establishing metabolic pathways or of identifying novel enzymatic reactions. With some exceptions this has been a relatively quiet area of science, usually peripheral to the main stream of biochemical research, and therefore not subject to much competition. Consequently, I have always worked in a rather relaxed atmosphere and have been able to enjoy several weeks vacation with my family each summer in the mountains, without developing a bad conscience for neglecting my students or suffering a fear of being scooped.

Most of the research embodied in my publications, particularly in my most productive years, was done by my students and postdoctoral associates. I have been fortunate in having many bright, enthusiastic, and dedicated collaborators, several of whom regretfully could not be mentioned in this chapter because of limitations of space. Much that we have accomplished is attributable to their skill and intuition.

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CURRICULUM VITAE

Horace A. Barker
Professor Emeritus, Biochemistry
University of California, Berkeley
April 1982

EDUCATION

Stanford University, A.B., Physical Science, 1929
Stanford University, Ph.D., Chemistry, 1933

EMPLOYMENT

Professor, University of California, Berkeley, Agricultural Experiment Station, 1936-1959
Professor, University of California, Berkeley, Department of Biochemistry, 1959-1969
Visiting Professor, Stanford University Medical School, 1962
Chairman, UC Berkeley Department of Plant Nutrition, 1949-1950
Chairman, UC Berkeley Department of Plant Biochemistry, 1950-1953
Vice Chairman, UC Berkeley Department of Agricultural Biochemistry, 1958-1959
Vice Chairman, UC Berkeley Department of Biochemistry, 1959-1962
Chairman, UC Berkeley Department of Biochemistry, 1962-1964
Professor, Biochemist, UC Berkeley Department of Biochemistry, 1969-1975
Professor Emeritus, 1975-

MEMBERSHIPS

American Chemical Society
American Society of Biological Chemists
American Society of Biochemistry and Molecular Biology
Society of American Bacteriologists
Biochemical Society (British)
National Academy of Sciences
American Institute of Nutrition
American Academy of Arts and Science

HONORS, AWARDS

Sug. Res. Award of National Academy of Science, 1945
Carl Neuberg Award, American Soc. Europ. Chem., 1959
Borden Award in Nutrition, American Institute of Nutrition, 1962
Election to National Academy of Sciences, 1953
Guggenheim Foundation Fellow, 1941-42, 1962
Sc.D. (honorary), Western Reserve University, 1964
California Scientist of the Year, 1965
Hopkins Memorial Medal and Lectureship, 1967
National Science Medal (Presidential Award), 1969
Faculty Research Lecturer, University of California, 1972
Honorary Membership in American Society for Microbiology, 1980

P R E S S R E L E A S E

Biochemist, National Medal of Science winner and retired UC Berkeley professor Horace A. Barker dies at 93

By Robert Sanders, Media Relations


Horace Albert Barker

Berkeley - Horace Albert Barker, one of the preeminent biochemists of the mid-20th century and professor emeritus of biochemistry at the University of California, Berkeley, died Dec. 24 at his home in Berkeley after a brief illness. He was 93.

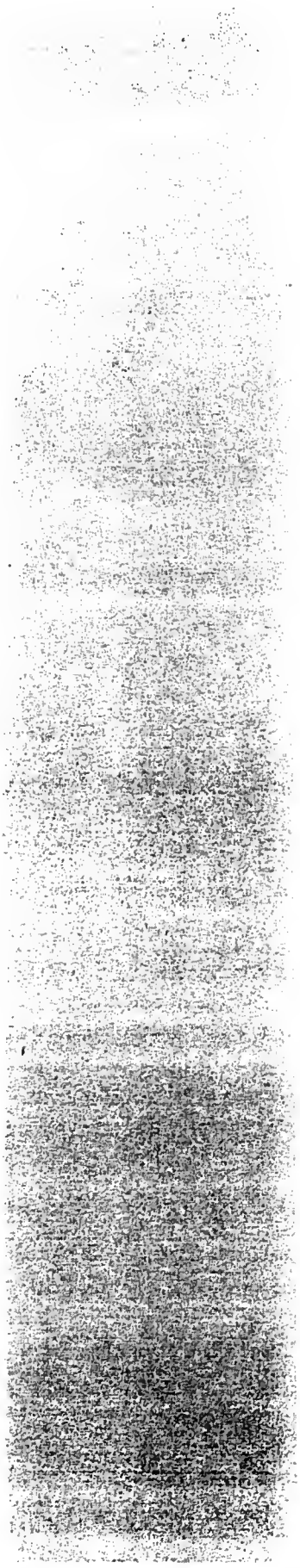
Barker, who had a research building named after him at UC Berkeley in 1988, is best known for work in the late 1950s on the biochemical function of vitamin B-12. This was regarded as a major advance in understanding the complex chemical conversion processes inside living organisms.

"He was richly deserving of the Nobel Prize for his work on coenzyme B-12," said biochemist Jack F. Kirsch, professor of molecular and cell biology at UC Berkeley. "He was one of the finest microbiologists who ever lived."

Barker also was a member of the team that, in 1944, first discovered the enzymatic steps living cells take when they synthesize sucrose - common table sugar. This feat involved one of the first uses of radioactive carbon-14 tracers, which Barker helped pioneer.

His studies in vitamin chemistry, bacterial metabolism, fatty acid oxidation and synthesis, carbohydrate transformations and amino acid and purine metabolism form a basic structure for much of our current understanding of metabolism and its role in sickness and health.

"He was a true leader in biochemistry and a leader on campus, widely respected internationally and by his Berkeley colleagues," said Daniel E. Koshland Jr., professor emeritus of biochemistry at UC Berkeley. "He was very self-effacing, but was the real core of the biochemistry department initially and as it developed on campus."



Barker won numerous awards for his achievements, including the National Medal of Science in 1968 and election to the National Academy of Sciences and the American Academy of Arts and Sciences.

An avid fisherman and outdoorsman, he continued into his 90s to hike and fish at his summer cabin near Mount Lassen.

Barker was born in Oakland, Calif., on Nov. 29, 1907, and raised in Palo Alto. He received his AB in physical sciences in 1929 and a PhD in chemistry in 1933 from Stanford University. After two years as a National Research Council Fellow at the Hopkins Marine Station in Monterey, Calif., and a year at the Technical University of Delft, Holland, he came to UC Berkeley as an instructor in soil microbiology in 1936. He was appointed a full professor of soil microbiology in 1946, but switched titles periodically until 1959, when he became a professor in the new Department of Biochemistry in the College of Letters & Science.

He chaired the Department of Plant Nutrition from 1949-50, the Department of Plant Biochemistry from 1950-1953, and the Department of Biochemistry from 1962-1964. He retired as a professor emeritus of biochemistry in 1975, although he remained active in the department into his 80s.

During his early career he studied the metabolism of ethyl alcohol and acetic acid (vinegar) in bacteria, providing important new information on the formation of fatty acids. Other bacterial studies laid the foundation for our current understanding of the role of folic acid, one of the B vitamins.

It was during work on a common soil bacterium, Clostridium tetanomorphum, isolated from the mud of San Francisco Bay, that he and his coworkers discovered in 1959 vitamin B-12 coenzyme - the active form of vitamin B-12 that performs certain critical chemical conversions in the body. He subsequently mapped out many of the metabolic reactions involving vitamin B-12 coenzyme, clarifying its role in building body tissue. This contributed greatly to an understanding of several human diseases, including pernicious anemia, caused by a deficiency of vitamin B-12.

Among the awards he received during his career were the Sugar Research Award of the National Academy of Sciences in 1945, with Michael Doudoroff and William Z. Hassid, his colleagues in the enzymatic synthesis of sucrose; the Carl Neuberg Medal of the American Society of European Chemists; the Borden Award in Nutrition from the American Institute of Nutrition in 1962; the California Scientist of the Year award from the California Museum of Science and Industry in 1966; and the Gowland Hopkins Medal of the London Biochemist Society in 1967.

He was elected to the National Academy of Sciences in 1953, authored or coauthored some 235 scholarly publications, and received honorary doctorate degrees from Western Reserve University, now part of Case Western Reserve University, and Munich University in Germany.

He is survived by two daughters, Barbara Friede of Piedmont, Calif., and Betsy Mark of Lexington, Mass; a son, Bob Barker of Camino, Calif.; and four grandchildren. His wife, Margaret (McDowell) Barker, died in 1995.

###

H. A. BARKER

1907-2000

H. A. BARKER

1907–2000

Professor Barker was born in Oakland, CA, where he lived until 1918 when his family moved to Palo Alto. He attended Stanford University and obtained a Bachelor's degree in Physical Sciences in 1929 and a Ph.D. in Chemistry in 1933. While at Stanford, he met and married Margaret McDowell, with whom he had three children, Barbara Friede of Piedmont, CA, Betsy Mark of Lexington, MA, and Bob Barker of Camino, CA. During a two-year postdoctoral at The Hopkins Marine Station he became interested in microbial metabolism, and this subject became the focus of his scientific career. After a year at the Delft Microbiological Laboratory in Holland, he joined the Berkeley faculty in 1936 as a Professor of Soil Microbiology. Subsequently, he held appointments in Plant Nutrition, Plant Biochemistry and, starting in 1959, in Biochemistry, and he served as chairman in all three departments. Barker's major research activities dealt with anaerobic fermentation by bacteria. He elucidated a general pathway for the formation of methane from carbon dioxide, acetate and methanol, and in so doing he pioneered the use of carbon-14 as a biological tracer. Using similar techniques, he demonstrated the reductive incorporation of carbon dioxide and ethanol into short-chain fatty acids and various amino acids. Turning his attention to the fermentation of amino acids, he uncovered new pathways for their decomposition that, with glutamate as a substrate, involved a novel chain rearrangement. This reaction was found to be dependent on vitamin B-12, which led Barker and his students to the landmark discovery of the coenzyme forms of the vitamin and to the discovery of other coenzyme B-12-requiring reactions. Barker received numerous awards, including the 1965 Borden Award in Nutrition, the 1966 California Scientist of the Year Award, and the National Medal of Science in 1968. Nook, as he was known to his friends and colleagues, was a role model and hero to several generations of life scientists. In 1988, the UC Regents renamed the Biochemistry Building H. A. Barker Hall in recognition of his scientific achievements and contributions to the University.

A MEMORIAL CELEBRATION
OF THE LIFE OF

H. A. BARKER

1907-2000

May 10, 2001
4:00 to 6:00 pm

THE GREAT HALL OF
THE MEN'S FACULTY CLUB

Music:
David Requiro, Cello
J.S.Bach Suite for Solo Cello No. 4 in E-Flat Major
S.Prokofiev "Children's March" for Solo Cello

Speakers:
Michael R. Botchan, U. C. Berkeley
Stuart M. Linn, U. C. Berkeley
Karen Mark, Harbor General Hospital, Los Angeles
Eric E. Conn, U. C. Davis
H.P.C. Hogenkamp, University of Minnesota
Herman J. Phaff, U. C. Davis
Paul K. Stumpf, U. C. Davis
Earl R. Stadtman, National Heart, Lung, and Blood Institute
Gerhard Gottschalk, Institut für Mikrobiologie & Genetik, Göttingen
Robert L. Switzer, University of Illinois
C. C. Wang, U. C. San Francisco
J. B. Neilands, U. C. Berkeley
D. E. Koshland, Jr., U. C. Berkeley
Robert Tjian, U. C. Berkeley

Co-organizers: **Michael R. Botchan**, Head of the Division of Biochemistry and Molecular Biology
Stuart M. Linn, Professor of Biochemistry and Molecular Biology

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Sally Smith Hughes

Graduated from the University of California, Berkeley, in 1963 with an A.B. degree in zoology, and from the University of California, San Francisco, in 1966 with an M.A. degree in anatomy. She received a Ph.D. degree in the history of science and medicine from the Royal Postgraduate Medical School, University of London, in 1972.

Postgraduate Research Histologist, the Cardiovascular Research Institute, University of California, San Francisco, 1966-1969; science historian for the History of Science and Technology Program, The Bancroft Library, 1978-1980.

Presently Research Historian and Principal Editor on medical and scientific topics for the Regional Oral History Office, University of California, Berkeley. Author of *The Virus: A History of the Concept*, Sally Smith Hughes is currently interviewing and writing in the fields of AIDS and molecular biology/biotechnology.